ACTA RADIOLOGICA
SUPPLEMENTUM VIII

THE EFFECT OF ROENTGEN IRRADIATION ON THE INTERRELATION BETWEEN MALIGNANT TUMORS AND THEIR HOSTS

A STUDY

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C. LUNDSGAARD
h.a.dec.

To the Memory of

my Father

Carl Elias Bonaventura Krebs, Ph. D.

born July 14th, 1851 — died December

22nd, 1921

FOREWORD

The investigations detailed in the following pages were carried out in the Roentgen Department of the Aarhus Municipal Hospital, during the years 1923 to 1927.

That it has been possible to undertake this experimental work is due to the liberality of the Aarhus Town Council, and particularly to the enlightened understanding of its Committee on Hospitals — Messrs. MOUSTEN, FALK and SALLING — who, realising that clinical work and experimental research ought to go hand in hand, have enabled me to establish a Laboratory of experimental Cancer Research in connexion with the Roentgen Clinic. For that, and for their never failing interest in our work, I am under great obligation to the Members of the said Committee.

For excellent help in carrying out this work of experimentation my best thanks are due to my former and present assistants, Dr. Jens JUUL and Dr. Aage WAGNER, to Head Nurse Miss BACH and to all the other members of the staff of the Roentgen Dep't.

To Prof. BOYSEN-JENSEN, Ph. D., to Miss Ingerid BREDSDORFF, to Prosector, Dr. GREGERSEN, and to Prof. Poul MØLLER, M. D., I express my grateful thanks for their valuable help and for the interest they have kindly shown in my work.

Aarhus, Denmark.
January, 1929.

CARL KREBS.

CONTENTS

Page

Introduction 9

Material 13

The Carcinoma.

The Sarcoma.

Earlier Transplantation Experiments 25

Technic of Transplantation 27

Author's Technic 29

Earlier Investigations concerning the Effect of Roentgen Irradiation on neoplastic Tissue *in vitro* and *in situ*, and the lethal Dose for such Tissue 31

Author's Technic of Irradiation 41

Author's Experiments concerning the Effect of Irradiation on Tumor Tissue *in vitro* and *in situ*, and the lethal Dose for such Tissue 42

The Influence of general Roentgen Irradiation on the Degree of Resistance to malignant Tumors 52

Earlier Investigations concerning the Growth of malignant Tumors in roentgen-rayed Tissue 60

Author's Investigations concerning the Growth of malignant Tumors in roentgen-rayed Tissue 67

Tables showing the Proportion of Takes, and the average and individual Duration of Life, in the various Experiments with Irradiation of Carcinoma and Sarcoma *in vitro* and *in situ* 97

Summary, (in Danish, English, French and German) 117

Literature 121

Plates.

INTRODUCTION

It was at about the beginning of the present century that the roentgen rays were first taken into the service of cancer therapy. At first, a great deal of concern was directed toward obtaining as short wave lengths as possible — partly in order to secure a strong penetration, partly so as to enable large doses to be given without lesioning the skin; the theory being that strong irradiation had a directly destroying effect on the cells of the malignant tumor. It did not take many years, however, before it occurred to the investigators that the effect of the rays was due not only to their direct action on the cells, but also to their action on the intercellular media.

Already in 1903 and 1904 it was tried — especially by some of the German scientists — to throw some light on the question experimentally; and little by little, as experimental pathology showed the unlikeliness of the therapeutical doses being able to kill the cancer cells directly, the theory of an indirect effect of the rays became more and more widely and generally accepted. At the same time, the clinical reports published from time to time showed that in several instances the results obtained by means of the large roentgen doses were far below expectations. The question therefore naturally arose, whether the reason for those unsatisfactory results might not lie in the fact that the rays — while probably stimulating to the natural defenses of the organism when used in small doses — became inhibitive, or even directly destructive, in their action on the latter when large doses were employed.

In Denmark, that idea was advanced by Thorkild Rovsing (197), who — at a meeting of the Society of Danish Surgeons, in

1919 — called attention to the fact that strong roentgen irradiation of a malignant tumor not infrequently seemed to result in an increased tendency to metastasis formation. It is especially in connexion with the results of prophylactic roentgen treatment after operations for cancer of the breast that this tendency has been noticed; probably because this type of cancer is particularly easy to follow in its development, both when it recurs and when it metastasizes; and perhaps also because both its recurrences and metastases are accessible and susceptible to treatment by roentgen.

The first considerable work dealing with this problem was a monograph by PERTHES (181), and this has in the course of time been followed by many others by various writers. The results to which they have come seem to be widely different. Most of them appear to have obtained the best effect from doses of medium strength, others with large doses; while a few have got the best results when they did not irradiate prophylactically at all.

PERTHES's (181) communication appeared in 1920. It comprises 362 cases. He and his assistant, NEHER (170) — who has made further studies on the basis of the same material — are among those who believe that the best results are obtained by refraining from prophylactic irradiation altogether. They found that intensive irradiation gave the poorest results, while a single, or several times repeated »inadequate» treatment had a moderately satisfactory effect. By »intensive» treatment, PERTHES understood two thirds of the skin erythema dose (2/3 HED) given through 3 mm. of aluminum on three fields: the chest, the axilla and the supraclavicular region altogether five times, at intervals of four weeks. By »inadequate» treatment he understood 1/2 of the erythema dose give through 3 mm. of aluminum. The question whether an operation for cancer of the breast should be followed by prophylactic irradiation or not, lies beyond the scope of the present work; it will be sufficient to mention a few of the widely divergent opinions successively advanced on the subject, and their authors.

Among the investigators whose results point in the same direction as those of PERTHES (181) and NEHER (170), are TIETZE (231), TICHY (230), KAESTNER (96), KOTZENBERG (108), LARSÉN and LYSHOLM (112), C. M. ROVSING (196) and EIKEN (52). One of the most recent contributions is the one by LARSÉN and LYSHOLM, who, on the basis of a fairly large material, reach the conclusion that recurrence after prophylactic irradiation is more frequent when large, single doses are given, than with the use of small doses several times repeated. The same opinion is held by EIKEN (52), while C. M. ROVSING (196) believes that cases which would fall under

groups II and III of STEINTHAL's (220, 221) classification[1] should be irradiated, while those falling under group I of that classification get along better if they are not subjected to roentgen.

HOFFMANN (82) has after-examined 388 cases. He expresses himself very guardedly concerning the results; still, he thinks that in cases belonging to the type of STEINTHAL's group II the use of large doses produced a certain improvement. He sums up his opinion in the words that: »die prophylaktische Röntgenbestrahlung hat vielleicht ein wenig genützt, geschadet hat sie den Kranken . . . nicht.» This is a far cry from the enthusiasm of LOOSE (127), who proclaims »Der Sieg der Röntgenstrahlen über den Brustkrebs», and reports 100 per cent. of cures in 55 cases which he treated by means of large doses — doses even larger than those which PERTHES called »intensive« irradiation.

LEHMANN and SCHEVEN (114), by using a technic somewhat similar to the one later preconised by PFAHLER (183, 184)[2], succeeded in increasing the percentage of cures from 32.7 per cent. to 47.5 per cent., for an observation time of three years.

ANSCHUETZ and HELLMANN (2, 3), who likewise form their opinion on the basis of a very large material, got their best results when using prophylactic irradiation. Their technic very much resembles the one employed by PERTHES; they believe, themselves, that the difference in the results may possibly be explained as due to a different classification of the material.

It will thus be seen that the diversity of opinion is very great. The question is certainly not one for me to try to settle. Nor have I any intention to attempt a comparison between the different results obtained — a matter presenting almost insurmountable difficulties anyhow, owing to the extreme variety of operative and roentgenological technics employed, the highly variegated material and divers manner of its classification. I have introduced the subject merely in order to show what led me into the study

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[1] STEINTHAL's grouping of the cases is as follows:

Group I: Tumors of slow growth, only a few centimeters large, and only adhesive to the skin. One or several nodules in the axilla.

» II: Tumors with visible growth; distinct adhesion to skin and pectoral fascia; distinct nodules in the axilla.

» III: The greater portion of the breast involved; extensive adhesion of tumor to the skin and underlying tissues; frequently also nodules in the supraclavicular fossa.

[2] PFAHLER thinks that the effect of the irradiation on the skin decreases according to a logarithmic curve, and that a very strong irradiation effect can be maintained for quite a considerable length of time, without causing injury to the skin, by giving a rather strong initial dose, supplemented with suitably graduated smaller daily doses afterwards.

of the problems on which I have endeavored to throw some light in the present work; namely:

1. How large a dose of roentgen is needed in order to produce a lethal effect on the cancer cell?

2. Is it possible to demonstrate any effect on cancer cells from doses that do not kill the latter?

3. Is it possible, by local roentgen irradiation, either to increase or to lower the resistance of an organism to a cancer tissue implanted either intracutaneously or subcutaneously in the exposed area?

MATERIAL

The Carcinoma

The first problem was to obtain the necessary tumors for use in the experiments. In order to answer my purpose, a tumor would have to be such a one as could be successfully transplanted through several generations; and, furthermore, it was desirable that it should be capable of yielding a fairly high percentage of takes. Formerly, at least, it was a more or less widely accepted theory that there was a rather considerable difference between carcinomata and sarcomata, in man, as regards their sensitiveness to irradiation; a theory which has found its concrete expression in the establishment — by SEITZ and WINTZ (216, 217), and by KROENIG and FRIEDRICH (110), among others, — of a separate »carcinoma dose» and »sarcoma dose». For that reason, I wished to work with a tumor of each kind, in order to test whether this theory of a different radiosensitivity applied with regard to the growth *in vitro* as well. I eventually succeeded in obtaining both a carcinoma and a sarcoma.

Whether the tumor was a spontaneous growth, or one produced artificially, I have considered as less essential. I have therefore, besides testing the serviceableness of the spontaneous tumors occurring in my animals, tar-painted a number of the latter, in order to obtain serviceable growths in that manner also. As animals for the experiments I chose mice; partly because they are not infrequently affected with spontaneous tumors, partly on account of the ease with which tumors are produced in them experimentally. In order not to miss any opportunity of obtaining the largest possible number of serviceable spontaneous tumors, I arranged with my »mouse-farmer» to send me, as far as possible, all the mice in which he discovered a tumorous growth of any kind. In that manner I succeeded in finding, among a total of about one thousand animals, 15 with spontaneous, malignant tumors. The fact that the question of the capacity of mouse-tumors for continued growth after transplantation had already been most ably and thoroughly dealt with

by other investigators, made it possible for me to leave that side of the subject alone, and to turn at once to the examination of those other problems which I have mentioned in the Introduction as soon as I had succeeded in obtaining a tumor suited to the purpose.

Such knowledge as we possess with regard to the anatomy of the tumors in mice is chiefly the result of research work done during the present century. Also communications of an earlier date exist, however, dealing with this subject in connexion with experimental investigations. Among them should be mentioned works by Morau (136, 137), Livinghood (122), Ebert and Spude (48), C. O. Jensen (89), Borrel (26, 27), Haaland (67, 70); Bashford, Murray and Cramer (17); Michaelis (133, 134), Apolant (6, 8, 9, 10), Murray (141), Lewin (118), Clunet (35, 36); Lynch (128) and Jobling (93). The first histological description of an adenocarcinoma in a mouse was given by Morau (136), in 1891. After that came the description, by Livinghood (122), of five spontaneous tumors which he believed to be adenocarcinomata, one being from the lung, the others from the breast. Later, Ebert and Spude (48) described the familiar occurrence of malignant tumors in mice occupying the same cage. They believed them to be endotheliomata; but from the description they might very well look to be adenocarcinomata. C. O. Jensen (89) described an adenocarcinoma with cystic alterations; without particularly dwelling on the purely anatomical aspects of the case, however. Michaelis (133, 134) described thirteen tumors, among which he distinguished three types: the alveolar — or tubular — carcinoma, the true adenocarcinoma, and a third type which, from the description, most nearly resembles a malignant adenoma. He believes that they all arose from the breast, and he mentions as a possibility that the last named might perhaps be an endothelioma.

Borrel and Haaland (67, 70), in their investigations, both worked with the same material, comprising altogether thirty spontaneous tumors. Besides the types already mentioned, they found an epithelioma of the lower jaw, a peculiar growth which they call a »molluscoid», and a case of infectious tumor-transformation of lymphoid tissue. Somewhat later came a description, by Bashford, Murray and Cramer (17), of an adenocarcinoma of the intestine. Altogether, only about seventy cases of spontaneous tumors in mice had been described up to the time when Apolant's (6) fundamental work appeared, in 1906. Afterwards, when interest in the question had become more general, they were found to be much more common than hitherto supposed. Thus, in Ehrlich's Institute about one thousand cases were observed during the ten years from

1904 to 1914. It is also noticed that the more recent writers are able to present a much more ample material as basis for their observations.

In his work from 1906, APOLANT (6) describes the epithelial tumors in mice. He has examined, in all, 276 individual tumors, from 221 animals, 207 of which were white mice, 14 grey. All the animals were females. This fact, that tumors never — or at least very rarely — are found in male mice, is borne out by the statements of other writers as well. APOLANT called attention to another peculiar fact; namely, that nearly all the tumors were seated on the belly-side of the animal, or at least in situations which made it logical to infer that they had arisen from that side of the body. It was the case with 259 of the tumors examined by him; and the observation agrees very well with those recorded by MURRAY (141) in his excellent work, published by the »Imperial Cancer Research Fund».

According to APOLANT, the tumors that occur spontaneously are partly benign, partly malignant. His list is as follows:

Benign: Simple adenoma,
simple cystadenoma,
cystic, edematous (or: hemorrhagic) adenoma,
papillary cystadenoma.

Malignant: Simple alveolar carcinoma (*incl.* hemorrhagic cystocarcinoma), papillary carcinoma (*incl.* the fissure-forming carcinoma).

APOLANT believed that all carcinomata in mice arose from the mammary region. In support of this belief he pointed, in the first place, to their histological structure, and furthermore to the fact that they were invariably situated in places where mammary tissue is to be found; that they were not adherent to the skin; that no signs of keratinisation were ever observed either in the original tumor or in the transplanted fragments; and, finally, that the tumors composing his material had all been found in female mice, and in most cases — as regards the young tumors, at least — completely surrounded by mammary tissue. Similar arguments were advanced by MURRAY (141) and LEWIN (118). It might perhaps be possible to mistake the tumors for carcinomata arising either from the sebaceous glands or from the basocellular layers of the epidermis; but the appearance and arrangement of the cells speak absolutely against the former suggestion; and, furthermore, the tumors would in that case be situated in the epidermis itself; and the same would be the case if they were basal-cell carcinomata.

With regard to the most anteriorly situated tumors it was not possible *a priori* to exclude the idea that they might have their origin from either the parotic or the submaxillary glands; nor, as regards the most posteriorly situated ones, that they might be arisen from the preputial glands, which are rather strongly developed in mice. But inasmuch as all those glands resemble the sebaceous ones very much, both in structure and in function, it would be reasonable to expect, also here, a difference in situation and appearance that would set them apart from carcinomata of the mammary region. As a matter of fact, the anterior tumors were often situated in very close apposition to the aforenamed mucous glands; but in all the cases in which the microscopical preparation included such a gland, APOLANT was able to show that there was a distinct separation between that and the tumor.

MURRAY (141), as we have seen, agrees with APOLANT (6) that the great majority of spontaneous tumors in mice originate from the mammary tissue. He has himself described altogether 142 tumors in the mammary region, from 119 mice. Most of them were similar, in structure and manner of growth, to the ones described by APOLANT; but MURRAY holds a somewhat different view with regard to the manner of origin of the various forms. Besides the true mammary carcinomata, he found in the region indicated: one angiosarcoma, one osteochondrosarcoma and three squamous-cell carcinomata. Besides those, he describes, in the same work, a number of tumors of other types, observed partly by himself, partly by other authors. He thus mentions: carcinomata of the small intestine, a spindle-cell sarcoma in the region of the kidney, adenomata and adenocarcinomata of the lungs, adenomata of the sebaceous glands and the liver.

In his work on the malignant neoplasms, CLUNET (35) has described, among others, eleven spontaneous mouse tumors, one of which was an adenoma of the sebaceous glands, while the ten others were tumors of the breast, some of them corresponding to the types described by APOLANT.

A wealth of different types are presented in the work by HAALAND (70), from 1911. His material comprises no less than 353 tumors, from 288 mice. The largest number, by far, were carcinomata of the breast, of which he had 325, fourteen of which were squamous-celled carcinomata. Of the others, 8 were squamous-celled carcinomata arisen elsewhere, 5 were carcinomata of the sebaceous glands; 2 carcinomata of the preputial glands; 2 adenomata of the kidney, one of them perhaps a hypernephroma; 2 adenocarcinomata of the ovaries; 6 sarcomata (four spindlecelled, one

roundcelled and one polymorphous-celled); 1 »mixed» tumor; 1 melanoma and 1 fibromyoma. Besides those, he had 30 adenomata of the lung, from 19 mice, and 21 lymphomatous tumors.

JOBLING (93) has described 41 spontaneous tumors; 29 of which were in the mammary region and corresponded exactly — as regards the microscopical picture — with APOLANT's description of the tumors in that region. Besides those, JOBLING describes, in the same work, 2 sarcomata, 1 papillary cystadenoma from the lung, 2 lymphosarcomata, 1 ovarian tumor, and a formation resembling a tumor and recalling the nodules seen in cases of molluscum contagiosum.

The spontaneous lung tumors which, under the microscope, can very much resemble metastases, have been described at length by LYNCH (128) and TYZZER (235).

Metastasis from those spontaneous tumors is by no means rare. APOLANT (6) found six instances of it among his 221 mice: five to the lung only, and one to the liver and spleen. MURRAY (141) found metastasis to the lung in twenty-seven, and to the lymph nodes in five, of altogether 68 mice. HAALAND (70), in 278 mice, found 103 cases of metastasis; four of which were in the liver, one in the kidney, and many in the lung. JOBLING (93) found metastasis to the lung in five out of 29 mice. For comparison it may be noted that metastasis from implanted tumors is relatively rare. Thus CLUNET (35), for instance, found only two cases of it among 230 animals; and, in another experiment, not a single case among 148 animals inoculated with cancer. It is believed that metastasis may occur as well through the blood streams as through the lymph channels.

With a few exceptions, all the aforenamed writers concur in the opinion that the microscopical anatomy of all these growths, as well as their manner of development and metastasis, make it indubitable that they are true malignant neoplasms — sarcomata and carcinomata — and that most of the spontaneous tumors in mice, by far, are adenocarcinomata arising from the mammary region.

All the tumors that occurred in my own animals, whether spontaneous or artificially produced, were transplanted subcutaneously into other mice. A great many of them grew successfully but died out after a few generations.

At last, on May 22nd, 1924, I found in the right side of the abdomen of a female mouse (no. 742) a tumor, about as large as a walnut, which gave positive result under continued transplantation, and which has been kept alive up to now, through more than seventy generations. It was not adherent either to the skin or to the tissue

underneath, and could be easily extirpated. Microscopically resembled what APOLANT designates as a »simple adenocarcinoma still, I have observed in some of the growths resulting from i transplantation a rather considerable cystic dilatation of the alveol The original tumor showed, under the microscope, an alveola structure, chiefly cellular, with a rather delicate connective-tissu stroma. The tumor cells formed islands of different sizes, and ana stomosing strings. Many of the cell-groups were solid; but i other places there were distinct glandular formations, roundis or of irregular shape, their epithelium either stratified or consistin of a single layer. The tumor cells were small, cuboid or rounc with a round or ovoid nucleus, the chromatin of which usuall lay more or less peripherally, thus giving to the whole nucleus vesicular aspect. The protoplasm was scanty, the character of th cells epithelial; they showed no tendency to keratinisation; mitose were few. There was no basal membrane underlying the stroma and the latter was in many instances so highly edematous that th tissue was hardly visible. Nevertheless, the alveolar arrangemen of the epithelial cells was perfectly distinct. Necrosis and inflam matory infiltration with leucocytes existed to a greater or lesse extent, and in various degrees of intensity. The growth of th tumor tissue was indubitably infiltrative.

The microscopical picture of the metastases in the lungs wa similar to that of the primary tumors. In many instances the lume of the vessels was filled with tumor elements, sometimes rolled u so as to form veritable emboli.

Macroscopically, the transplanted tumors were of a rather firm consistency; their surface slightly nodular, in many instances formin distinct lobules. Most of them were spherical; in not a few of them there were cystic cavities containing a serous, sometimes slightl hemorrhagic fluid. Only in a few cases was the growth visibl infiltrative; but especially among the old animals there would no and then be cases in which the tumor had grown through the ski and muscles; in one instance it had grown through the peritoneum When the carcinoma had been transplanted subcutaneously int the breast of a mouse, it was possible to detect the first signs o a small tumor ten or twelve days after; sometimes a little later The carcinoma could develop to a size far exceeding that attaine by any sarcoma. Thus, I have had animals that weighed 42 grams 20 grams of which were represented by the tumor. It would, how ever, take from three to four months for the growth to become a large as that.

The length of time that the »carcinoma-mice» would live, fro

the day the transplantation took place, varied conspicuously. Nevertheless, it was entirely possible to use the average duration of life as a measure for the effect obtained from an irradiation.

The average of takes from transplantation was about 50 per cent.; though the proportion might vary from about 40 per cent. to about 60.

During all the time while the experiments described in the following were being carried out, there was no lasting increase or decrease in the proportion of takes; but since that time it would seem as if the tumor has become divided into two strains, one less virulent, the other more. Metastases occurred in 7 per cent. of the cases; macroscopically they have been demonstrated only in the lungs. In all cases where metastases are spoken of in the following, the diagnosis has been verified by microscopy.

The average duration of life of the mice with carcinoma was 64.6 days. The question of the importance of this point will be dealt with farther on.

The Sarcoma

The increasing frequency with which cancer in man was observed to develop as the result of either chemical, thermic, mechanical or actinic irritation, led to a line of experimentation being started, toward the end of the last century, with a view to finding out whether those same conditions could be produced experimentally. The first observation of the kind alluded to was made, as early as in the latter part of the eighteenth century, by PERCIVAL POTT (185), who conceived the idea that the frequent occurrence of cutaneous cancer in chimney-sweeps might be due to the action of the soot on their skin. That this affection was particularly prevalent precisely among the English sweeps was due to the fact that English chimneys, more than others perhaps, contain a great proportion — if not all — of the products resulting from the destructive distillation of anthracite coal, which is the fuel almost exclusively used in that country. The distillation takes place in the heated upper layers of the coal with which the grate is filled, before these have yet caught fire from the burning layers beneath; and there is thus a possibility for the products released in the process to be carried up into the chimney, where they are deposited together with the soot. The latter may possess certain cancerogenic properties similar to those residing in various kinds of tar. At least

it is a curious fact that chimney-sweeps' cancer is very rare in Sco land, where the same type of chimneys are used as in Englan only the fuel being a different kind of coal. That the cancerogeni power of the tar depends in a large measure on its compositio has been proved experimentally by DEELMAN (42, 43). That als other conditions, such as temperature, for instance, have a certai bearing on the matter would seem to appear from the investiga tions of BANG (13). For the whole work of experimental cancer re search, POTT's (185) observations became of the highest importanc because they led to experiments being started with a view to settlin the question of the cancerogenic properties of tar and soot; expe iments which especially during the last fifteen years have con tributed materially to throwing light on certain sides of the cance problem.

One of the first communications dealing with an attempt t produce cancer by means of tar was made by HANAU (75), in 188 He painted the scrotum, vulva and nipples of cats with the sub stance; but nothing resulted except dermatitis and a thickening o the epithelium involved. Since then, a long series of communica tions have recorded vain attempts in the same direction, both wit the use of tar and other distillates. Among those who have worke with the problem are BROSCH (30), HAGA (72) and HAXTHAUSE (77). The last-named tried with anilin, para-amidophenol, ben zidine, and with a 10 to 20 per cent. naphtylamin ointment; bu he relinquished his experiments after only four weeks, which ma be the reason why he did not succeed in producing cancer in an of his animals.

The first communication of any positive result in this directio appeared in 1915, from Japan, where YAMAGIWA and ICHIKAW (256, 257, 258, 259, 260) — inspired, as they state themselves, b the first of FIBIGER's (54) famous investigations concerning sp roptera cancer — commenced a series of experiments aiming a producing cancer in rabbits by painting the ears of the animal with coal tar. The experiments were taken up by TSUTSUI (234) with mice; with the result that he succeeded in producing metasta sizing carcinomata and, in one case, sarcoma. The following yea YAMAGIWA and ICHIKAWA (259) were able to report that they ha succeeded in producing in three mice the initial stages of cancroi of the mamma, in a fourth one a fully developed cancroid, and i a fifth one a transplantable myxofibrosarcoma — all of them b injection of tar-lanolin in the mamma. By the same method, SEE DORFF (215) succeeded in producing adenocarcinoma of the breas in a rabbit and a white mouse; the latter of which gave metastase

When the report of those experiments reached Denmark, FIBIGER and BANG (55) set to work, at the Institute of Pathological Anatomy of the University, to try whether they could provoke malignant tumors in mice by painting them with tar. Jointly they made two sets of experiments — the first series comprising 15 white mice, the second one 10; and simultaneously BANG commenced a third series, with 20 mice.

The animals were painted with coal-tar every second or third day, in exactly the same spot of the skin, between the scapulæ. In 12 of the twenty-five mice comprising the two first series the painting failed to produce any pathological changes that could with any degree of certainty be interpreted as cancer. In 3 there came only a papilloma, accompanied by some inflammation; but of the remaining ten, 8 developed a malignant epithelioma with or without keratinisation, and 2 a carcinosarcoma.

The cutaneous changes produced by the tar-painting were rather similar in all the animals, at least in the early stage. It generally began by the hairs falling out; later the skin would become more or less thickened and rough; in some cases there would be a formation of crusts and fissures. In several instances the skin eventually became smooth again afterwards. In all the animals one or more small, wart-like tumors appeared after various lengths of time, some as early as four months, others as late as eight months after the first painting. Usually, the papilloma would eventually fall off, and would then be replaced either by an ulcer or by a fresh papilloma, the growth of which would be rapid, so that in a few months' time it sometimes became as large as the head of the animal itself. Those fresh papillomata were covered with thick layers of keratinised cells, and the surface of the ulcers with crusts of dried-up secreta and horny, necrotic masses that sometimes attained an enormous size. In most cases the tissues surrounding the tumor or ulcer sooner or later became infiltrated, and as soon as this became evident the tar-painting was ceased with. In this manner FIBIGER and BANG succeeded in provoking infiltration in 213 days. BANG (13), in the third series of the experiments alluded to, succeeded in producing cancer in 182 days.

FIBIGER and BANG tried transplantation of three of the malignant epitheliomata. With two of them the result was negative; the third one kept alive during four generations. They also tried transplantation of one of the carcinosarcomata, and in the course of one year — up to the time of publishing their results — they had succeeded in keeping it alive during thirteen generations. Me-

tastasis to the lymph nodes occurred in two of the animals in whor the tar-painting treatment had provoked cancer.

In his subsequent experiments, BANG (13) observed an infiltra tive growth already within three months after the beginning c the treatment. DEELMAN (42, 43), however, by a combination c scarification and tarring, obtained infiltration already within eigh weeks; and even more rapid were the results obtained with rabbit by ICHIKAWA and BAUM (85). By feeding the animals with lanoli and arsenic, respectively, simultaneously with the tarring treatmen they succeeded in provoking incipient cancer in 35 days, and full developed cancer in 47 days, from the first application of the ta

It is nearly always a carcinoma or carcinosarcoma that result from the tarring. There are also several examples, however, of th tar — or tar products — producing sarcomata; thus, the case c myxofibrosarcoma mentioned above, which was provoked by th injection of tar-lanolin in the breast. TSUTSUI (234) tells of a spindl cell sarcoma resulting from tar-painting; and RUSSELL (205, 206 got a spindle cell sarcoma and a polymorphous cell sarcoma, respec tively, in mice and rats, in the course of from eight to eleven months by weekly injections, into the subcutaneous tissues, of from 10 t 15 milligrams of tar. In the same manner, he provoked a trans plantable osteosarcoma in a rat after eighteen months; the tumo appearing 6 months after he had ceased with the injections. REH (193) describes a case of sarcoma in the vesica of a fuchsin-worke and ascribes its origin to the action of the anilin on the wall of th bladder.

In his work from 1924, BANG (13) explains in detail the manne in which the tar cancer arises and develops. He uses the term »biologically malignant» to indicate a cell that, to all appearance normal, may nevertheless become the starting point from which a malignant tumor arises. This condition of biological malignancy of ten sets in long before the cell begins to exert its malignant action In mice it needed at least two months of tar-painting to produce it BANG further showed that a tarring period of thirty days is no sufficient to produce cancer, while the continuation of the treatmen for 120 days gave positive results in 100 per cent. of the cases The time from the beginning of the treatment to the moment o incipient infiltration he calls the »hatching time»; and this he divide into two periods: the »period of reaction» — by which he under stands the time required to render the tissues biologically malignan — and the »period of latency», which is the time from then unti the moment the histologically demonstrable development of th tumor begins. He found that the shorter the period of reaction

the slower the development of the tumor; and reversely. This is in agreement with the already previously published observation, by MURRAY and WOGLOM (144), to the effect that it is possible to obtain a demonstrable cancer from the transplantation of cells not yet histologically malignant. MURRAY and WOGLOM transplanted one half of the base of an apparently benign papilloma — produced by tar-painting — and after some time a carcinoma developed on the site of the grafting.

To me, the most interesting thing about BANG's experiments is his demonstration of the fact that a mouse that has been painted with tar for a certain period may develop cancer after a shorter or longer lapse of time without necessarily having shown signs of anything abnormal in the interval.

Since the publication of the Japanese and Danish reports there have appeared a number of other communications concerning the cancerogenic power of tar. MAISIN and PICARD (130), for instance, describe how the injection of tar into the vesica of a rat produced cancer in that organ. Reports dealing with the effect of tar on the skin have been made by ROUSSY, LEROUX and PEYRE (195), JORDAN (95), HALBERSTAEDTER (74), DOEDERLEIN (47), YAMAGIWA, FUKUDA, KANEKO and AZUMA (261), MAISIN and DESMEDT (129), MURRAY (143) and several others. As regards the macroscopical and histological picture, the description given by all those writers agrees very largely with the observations of FIBIGER and BANG.

In December, 1924, I began to tar a number of mice that — for another purpose — had been roentgen-rayed on the hind-part of the body, the roentgen dose being 2 Sabouraud & Noiré through 5 mm. of aluminum at 23 cm. Following the directions of BANG and FIBIGER, I painted between the shoulder-blades 3 times a week for six months; and during that time I observed all the same changes as described by them. Eventually, not a few of the animals developed malignant tumors; but only one of these — from mouse no. 847 — grew when transplanted.

As seen under the microscope, this tumor proved to be extraordinarily rich in cells. They were decidedly polymorphous, and the stroma very scanty. Indian-file arrangement of the cells was very slight; and in a few places the spindle-cell type would then be preponderant. Most of the cells were either round or polyhedral; they were of various sizes, but mostly large. The protoplasm was scanty; the nuclei large, either oval, round or irregularly shaped, with chromatin strongly stained and of various design. Here and there, scattered through the tumor tissue, were numerous very large

cells, mononuclear or polynuclear — like larger or smaller giant cells — the shape, both of the cells and the nuclei, being irregular. In all of the tumor tissue there were numerous mitotic figures. The slightly apparent stroma consisted of small blood vessels and fine collagen fibrils; these elements being diffusely distributed in direct contact with the tumor cells. There was not even the slightest indication of any alveolar arrangement of the stroma. More or less scattered throughout the tumor were found striated muscular cells, indicative of the pronounced capacity of the tumor tissue for invasive growth. Many of those muscular cells were markedly degenerated. Finally, there were necrotic areas of various extent, with broken-down nuclei of cells and obliteration of structure. The picture of the lung metastases was similar to that of the primary tumor. Neither in the metastases nor in even the most long-lived primary tumors was there ever found any sign of keratinisation.

As I deem it outside my task — and beyond my competence — to express a final opinion as to whether this tumor is a sarcoma, a carcinosarcoma or a true carcinoma — while, morphologically, it most nearly resembles a sarcoma — I have, for the sake of expediency, used the latter designation all through, when speaking of this tumor. This is the same stand as the one taken by FIBIGER and MOLLER (56) with regard to their experimental tumors, when they say that: »in planning and performing these investigations we have not intended to subject to any quite exhaustive examination the much discussed question whether or not experimental tar tumors are only pure carcinomata or may be carcinosarcomata or sarcomata as well; an examination that would require special and extensive researches which, up to the present, we have not been able to perform.»

In contrast to the carcinoma, the sarcoma had a very marked tendency to grow through skin and muscles. Ulceration was not infrequent, and sometimes its growth would extend all the way through the thorax and the abdominal wall, out into the pleura and peritoneum. The surface of the tumor was rather smooth, sometimes more or less rugged; its consistence rather uniformly firm all through. The central parts of this tumor showed a less pronounced tendency to cystic changes than did the carcinomata. When grafted subcutaneously, it grew very rapidly. As a rule, there would be a small, palpable tumor already after ten days' time; in fifteen days it would have become as large as a good-sized pea, after about thirty days it usually covered the greater part of the thorax; and by that time most of the animals died. When the grafting was done

intracutaneously, the animal would, on the average, live for about ten days longer. The sarcoma implanted in the subcutaneous tissue gave metastasis in about 20 per cent. of the cases; while, with intracutaneous implantation, the frequency of metastasis would be as high as 60 per cent.; possibly for the simple reason that the animals then lived longer. Just as with regard to the carcinoma, we are here speaking only of macroscopically visible metastases verified by microscopy.

EARLIER TRANSPLANTATION EXPERIMENTS

Apolant's (10) monograph on experimental tumor transplantation, already referred to, contains a record of all the experiments in that line, from Peyrilhes' publication in 1773 up to 1913. A survey of the literature thus compiled shows that the possibility of transferring a tumor either from man to an animal or from one animal species to another must be regarded as extremely doubtful.

The list of attempts at heterologous transplantation is very long, and the result in most of them negative; still, a few of the writers believe that they have succeeded. There can hardly be any doubt, however, but that a great deal of what they took to be transplanted tumors were in reality abscesses, granulomata or spontaneous neoplastic growths in the animals experimented on (Lebert and Wuess (113); Duplay and Cazin (46); Apolant (10)).

Among the first who have reported results are Langenbeck (111), Klencke (101), and Lebert and Wuess (113). The first-named, after several unsuccessful experiments, partly with cancer pus, partly with cancer tissue, succeeded in provoking some small nodules resembling cancer, in the lung of a dog, by injecting into the veins of the animal the cancer juice from a recently removed medullary carcinoma of a human humerus. The nodules appeared two months after the injection had taken place.

From the years immediately before and after the beginning of the present century there are a great many communications on the same subject; thus, by Hanau (75), Moreau (139), Duplay and Cazin (46), Loeb (124, 126), Velich (237), Sticker (222, 224), Ehrlich (51), and later by Lewin (116), Strauch (225), Fischl (62), and Jobling (94). Some of these, like Lebert and Wuess (113), Hanau (75), Moreau (139), Sticker (224), Lewin (116), Fischl (62) and Jobling (94), have experimented with transplantation from man to animals. Lewin thinks he has seen positive

results from the transplantation of a human ovarial sarcoma to a dog, and JOBLING got a human, sarcomatous teratoma to grow for a few days in a monkey, until it eventually became resorbed. All the others have only negative results to report.

In the line of transplantation from one animal species to another, MOREAU thinks he succeeded in transplanting mouse tumors into sheep, and STRAUCH tells of a tumor with metastasis formation provoked in a rabbit by injection of mouse-carcinoma pulp. The tumor could not be transplanted back into mice, but could be transplanted on to other rabbits. EHRLICH showed that a mouse tumor could grow for a while in a rat, but that it became resorbed unless it was shortly transplanted back into another mouse, whence it might again be transplanted to a second rat, and would be able to keep alive there for a brief period.

Quite recently it has been shown, by MURPHY and STURM (156), that a heterologous tumor fragment may be successfully transplanted, when the transplantation is made to brain tissue in such a manner that it does not come into contact with the ventricles; but whenever such contact occurred, the usual reaction set in around the tumor, and it became resorbed.

A review of all those experiments conveys the impression that heterologous transplantations in any kind of tissue — with the only exception, perhaps, of brain tissue — succeed very rarely indeed, if they are possible at all. With homologous transplantations, on the other hand, better and better results are achieved toward the end of the nineteenth century. In that connexion it is only just that ALIBERT's (1) name should be mentioned — as the first who attempted to transplant tumor tissue from one human organism to another. His experiments, in 1808, of inoculating himself and some of his pupils with cancer juice produced nothing but inflammation in any of the subjects, however. Experiments in that direction have naturally been very few; those undertaken by HAHN (73), v. BERGMANN and CORNIL (23) are the only ones of which any reports exist. On the other hand, homologous transplantation from one animal to another is being more and more commonly experimented with. The first communication on that subject was made by NOWINSKY (172), who, in 1876, made a series of transplantations of medullary carcinoma from dog to dog — in 27 cases to inflamed skin, in 15 to fresh wound-surface. From the former there were no takes; from the latter, tumors developed in two of the animals, with metastasis to the lymph nodes. Those tumors were carried onward for yet another generation. Of others who have worked and reported on the same subject may be mentioned v.

Eiselsberg (49), Hanau (75, 76), Moreau (136, 137, 138, 139) Duplay and Cazin (46), Velich (237), Loeb (124, 125), Sticker (223) and, especially, C. O. Jensen (88, 89, 90, 91, 92). von Eiselsberg, Hanau, Velich and Loeb used chiefly rats for their experiments. Duplay and Cazin, as well as Sticker, transplanted from dog to dog; Moreau used mice; Jensen both rats and mice. The experiences as recorded by all these investigators have made it clear that dogs, at least, are poor subjects for transplantation experiments, while mice and rats are excellently suited to the purpose. Following the pioneer work done by the men already named, further experiments along the same lines have been carried out in a great many places; by Michaelis (133, 134), Loewenthal (135), Borrel (26, 27), Ehrlich (50, 51), Apolant (6, 7, 8, 10), Bridré (28, 29), Bashford (15, 16, 19), Murray (15, 16, 17, 18, 19, 142), Haaland (19, 68), Bowen (19), Gierke (66), Clunet (35, 36), Woglom (251) and many others.

TECHNIC OF TRANSPLANTATION

The methods of transplantation used by the early investigators were practically the same as the ones used to-day.

Hanau (75) and von Eiselsberg (49) simply transferred tumor fragments, which were placed in the desired position by the use of either a cannula or a forceps. Langenbeck (111) reduced the tumor tissue to a pulp, which he injected by means of a syringe. Moreau (139), Duplay and Cazin (46), Sticker (222, 223 ,224) and Jensen (88, 89, 90) used both those methods. Sticker (222), Moreau (138) and Loeb (124, 125) have also tried the injection of cell-free filtrates, but without success. Velich (237) injected blood and tissue-juice. Loeb (124, 125) obtained some positive results from the injection of cystic fluid from tumors; but he believes himself that it must probably have contained some tumor cells. Ehrlich (51) injected the substance in the form of an emulsion. Bashford, Murray, Haaland and Bowen (15, 19) used implantation both of tissue pulp and of fragments. Murray (141) and Gierke (66) preferred the latter, arguing that they would thus avoid, in the first place, injury to the stroma; in the second place, immunisation of the animal, which they believed might be facilitated by the introduction of the more easily resorbed emulsion. With regard to the quantity of tumor substance that ought to be transferred, Bashford, Murray, Haaland and Bowen (19) be-

lieved that the best results were obtained by using tissue fragments weighing between 0.005 and 0.03 grams. If emulsion was used, from 0.025 to 0.25 ccm. would be the most suitable quantity. BASHFORD (14) pointed out the necessity for letting a reasonable time go by before carrying a transplanted tumor onward. While some tumors can only be cultivated by means of quick transferences, he believed that in other cases he had observed a certain exhaustion of the tumor cells, when insufficient time was allowed between the individual transplantations. From this exhaustion the cells might again recover if they were allowed to remain for a sufficient length of time in one animal. I have also myself made the observation that fragments of old tumors grew more slowly than those from younger tumors, even under conditions as nearly as possible alike for both; I have therefore always taken care that the tumors used for transplantation should be, as far as possible, of the same age.

AUTHOR'S TECHNIC

My own proceeding for transplantation has been to graft the tumor fragments either subcutaneously or intracutaneously, using either a cannula or a forceps.

The animal from which the graft was to be taken was killed with ether, and the hair removed from the abdomen by means of a forceps, whereupon it was plunged first for two minutes into a 5 per cent. alcoholic solution of iodine, afterwards into two successive baths of 70 per cent. alcohol. It was then pegged out on an operating table covered with sterilised paper; an incision was made in the abdomen, distally from the tumor, and the skin over the latter gently loosened from beneath. The sterilised instruments used for the skin were discarded one after another, and with fresh, likewise sterilised ones the tumor was removed and placed in a sterilised Petri dish containing a little normal saline solution of chloride to prevent the tumor from drying up.

By means of scissors and forceps the tumor was next cut up into pieces as nearly as possible of the same size, each about 1 millimeter in diameter. With some practice it was possible to do this so exactly that it was difficult with the naked eye to see any difference in the size of the pieces. I was not so much concerned with finding out what size of fragment was preferable, as with getting fragments of equal size for all the control- and experimental animals.

When the implantation was to be done, the animal was anesthesised with ether, the hairs epilated from the right side of the abdomen, the spot disinfected by painting with iodine, twice repeated, and it was then pegged out on the operating table as already described. An incision about 1 centimeter long was made through the skin at the spot where the hairs had been removed, and while an assistant kept the edges of the wound apart a blunt instrument was pushed up into both axillæ, whereupon a small fragment of tumor was placed in each of these by means of a forceps. The wound was then closed with chromiodine catgut, and covered with collodium and cotton. In order to create conditions as equal as possible,

I implanted on experimental and control animals by turns, first five of one kind, then five of the other, and so forth.

Only in cases where the tumor tissue had been roentgen-rayed for a very long time *in vitro* did I deem it best to do the implantation on the control animals last, in order to make sure, as far as possible, that any deterioration perchance undergone by the tissue would be only such as might be due to the irradiation, and not to the — in those cases unavoidable — circumstance of its rather protracted existence outside the organism.

The intracutaneous implantation was done by making incision in the right side of the abdomen as before, and then loosening the skin from the fascia of the muscles underneath by means of a blunt instrument, whereupon a fine cannula, into which a small fragment of tissue had been aspired, was introduced into the skin from beneath. When the point of the cannula had reached the spot where it was desired to implant the fragment, the latter was pushed out from the cannula by means of a stylet. This done, the wound was closed and covered with collodium and cotton as described above.

In order to find out in what part of the skin the tumor fragment would become lodged by this proceeding, I examined several pieces of skin under the microscope immediately after the implantation had been done. In nearly all the cases I found the fragment lying immediately beneath the epithelium. Sometimes the platysma would be pushed to one side, and the tumor tissue rested on the fascia of the skin (see Plate III).

Afterwards, the animals were examined once a week; the tumor being measured and a tracing of its outline made on a mouse-diagram.

In two series of my experiments I did the same as was done by Murphy, Hussey, Maisin, Nakahara and Sturm (146, 148), and by Beck and Engel (20), in some of theirs; namely, left one side of the animal uncovered during the irradiation, in order to try whether it could be shown what influence the general effect of the latter might have on the tumor tissue of that side.

EARLIER INVESTIGATIONS CONCERNING THE EFFECT OF ROENTGEN IRRADIATION ON NEOPLASTIC TISSUE IN VITRO AND IN SITU AND THE LETHAL DOSE FOR SUCH TISSUE

It is the great merit of the Danish scientist, C. O. JENSEN, that he — after the possibility of homologous transplantation on a large scale had been proven — was the first to take up the study of that immensely interesting problem: the reaction of the tumor cells outside the organism. JENSEN thus became, in reality, the founder of that entire branch of modern biological research which deals with the biology of tumors, and which owes its impulse to his first reports (88, 89, 90), published in the early years of the present century.

It is probable that to future investigators the cultivation of neoplastic tissue *in vitro* may become just as important as JENSEN's work has been for us of the present generation. Also along that line of experimentation some valuable work has been done by a Danish scientist; namely, by Albert FISCHER (57, 58, 59, 60).

In my work I have used only irradiation in vitro, not *in culture*, and I have found that those characters of my tumors which were the object of my study did not alter even when the tissue remained outside the organism for quite a lengthy period.

The first investigations concerning the biology of tumors, as regards their susceptibility when detached from the organism, were directed especially toward showing the reaction of temperature, light and various chemical substances on the neoplastic tissue. Experiments to that effect were made by LOEB (125), MICHAELIS (133), CLOWES (40), STICKER (222), BASHFORD, MURRAY and CRAMER (16), EHRLICH (51), LEWIN (118), and HERTWIG and POLL (78). C. O. JENSEN showed that the neoplastic tissue preserves its vitality best at a low temperature. For instance, he succeeded in keeping tumor tissue alive for eighteen days, when keeping it at a temperature of from 1 to 3 degrees; while, if kept at room-temperature, it failed to take after twelve days. Temperatures of above

47° and below −18° would kill it in three minutes. In collaboration with Hans JANSEN (89) he showed that irradiation with concentrated Finsen light, alternately from either side, would kill a layer 0.2 mm. thick in from 1 to 2 minutes; while even a full hour's irradiation did not kill a layer of only 1 millimeter's thickness. Like JENSEN, LOEB and EHRLICH, as well as HERTWIG and POLL, found that the neoplastic tissue keeps best at a temperature around the freezing point, while high temperatures kill it more quickly. The rest of the writers named above have followed the same paths as JENSEN, and their results are more or less similar to his.

To me it has been particularly interesting that HAALAND (71), EHRLICH and APOLANT (10) have shown that the temperature may have a different influence on the two components of the same carcinosarcoma. For instance, HAALAND showed how the carcinomatous characters of such a tumor might be eliminated by heating for half an hour to 44°; and EHRLICH and APOLANT found that the sarcomatous element of a mixed carcino-sarcoma emulsion became weakened when the emulsion was exposed for seven days to a temperature of −10°. When the mixture was injected without having undergone this cooling, only a sarcoma developed; while, after the freezing had taken place, its injection resulted in the production of a true carcinoma sarcomatodes. Something of the same kind has been tried by FIBIGER and BANG (55) with experimentally provoked carcinosarcomata, but without their being able to confirm the results of the other investigators named.

It has long been known that certain sarcomata *in situ* are much more easily reactive to the rays than carcinomata. It might therefore be imagined that sarcoma- and carcinoma cells *in vitro* would likewise prove to be differently influenced by the rays, just as they are by the temperature; but of the two tumors with which I have worked, that has not been the case.

Of recent years especially, a certain amount of investigation has been going on concerning the action of radium and roentgen rays on tumor tissue *in vitro*. It is that part of the biology of tumors which I have particularly set out to study.

The first experimental work on the action of radium rays on neoplastic tissue, by APOLANT (4, 5), appeared as early as in 1904. It dealt only with irradiation of the tissue *in situ*, however. The first report concerning investigations into the similar action of roentgen rays did not come until some years later, from a Danish scientist, MEISEN WESTERGAARD (246). It dealt with the effect on the neoplastic tissue both *in situ* and *in vitro*. Meisen WESTERGAARD

used two strains of Jensen's mouse-carcinoma that gave takes in from 40 to 60 per cent. of cases, sometimes oftener. After excising the tumor he flattened it, pounding it in a sterilised mortar until it was only 5 millimeters thick; whereupon he cut it into two equal parts, each of which he placed in a Petri dish containing a small quantity of a normal saline solution. At first he used the process of implanting fragments by means of a cannula and stylet; but he soon abandoned that method and, instead, injected a few cubic centimeters of the tumor substance, shredded and mixed with a normal saline solution, under the skin. He does not seem to have laid weight on any particular exactness of dosage. For irradiation he used a Bauer tube of medium softness, no filter, and a distance of 14 cm. With that arrangement it took him 24 minutes to give 1 Sab. & Noiré; the strength of his current being 0.5 milliampère, the secondary tension 10 centimeters. Each time, he inoculated 16 mice with irradiated tissue and 16 others for control. In this manner he got the following results:

Irradiation time:		no. of takes:
30 min.	irradiated tissue	0
	non-irradiated »	7
24 »	irradiated tissue, with inhibited growth	1
	non-irradiated »	9
18 »	irradiated tissue, with belated growth	5
	non-irradiated »	7
12 »	irradiated tissue	11
	non-irradiated »	10

Beside determining the lethal dose for the tissue *in vitro*, MEISEN WESTERGAARD showed that doses of lesser strength than the lethal one may be inhibitory to the growth of the tumor; and he further believed that by irradiating a tumor immediately after its implantation he was able not only to retard its growth, but also to prolong its period of latency proportionally with the increased viability thus obtained. I shall come back, in the following, to the question as to what importance ought to be attached to the latter theory.

From 1910, we have two French reports dealing with the subject of roentgen irradiation of neoplastic tissue *in vitro*, by CLUNET (35) and CONTAMIN (41). CLUNET used a very virulent polymorphous-cell sarcoma. His experimental material comprised 32 mice, 16 of which were roentgen-rayed on the abdomen, over the area where the tumor tissue was subsequently to be implanted. The size of the area is not stated; the dose was of 20 H. The tumor tissue was divided into two portions, one of which was irradiated *in vitro*

previous to being implanted. For that purpose, the irradiation dose was of 30 H. A Chabaud's tube was used, the strength of the current 0.8 milliampères, the focus-distance 10 centimeters. For the animals no filter was used; the tissue *in vitro* was irradiated through the glass cover of a Petri dish as a filter. The experiments were four in number:

Exp. *I:*	8	non-irradiated	mice	inoculated	with	non-irradiated tissue
» *II:*	8	»	»	»	»	irradiated »
» *III:*	8	irradiated	»	»	»	non-irradiated »
» *IV:*	8	»	»	»	»	irradiated »

All the animals in *Experiment I* developed tumors that 4 days after the inoculation were as large as a pea, and after 20 days as large as a hazel nut. They all died between twenty-five and thirty days after being inoculated. Of the animals in *Experiment II* only one had developed a visible tumor after 4 days; but after 10 to 12 days each had a tumor as large as a pea, and after that the tissue grew more rapidly, so that by the 20th day the tumors of them all were just as large as those of the mice in Exp. I. All the animals of this second lot died within twenty-nine days after being inoculated. The animals in *Experiment III* all died within six days after being inoculated, without any tumor having appeared. Of those in *Experiment IV*, six died within 9 days from the inoculation. The two remaining ones did not develop any tumor; and when they were killed, thirty days after being inoculated, even the microscopical examination failed to reveal any trace of tumor tissue.

It will be noticed that the irradiation *in vitro* did not kill the tumor, but shortened its period of latency. It affected neither the average of life nor the proportion of takes. Clunet believes that the death of the animals in Experiments III and IV was not due to the roentgen treatment alone, but also to the harmful influence of the tumor tissue on their organism. That all the animals in Exp. III died, but only six of those in Exp. IV, he ascribes to the fact that, in the case of the latter, the tumor tissue had become enfeebled as a result of being irradiated *in vitro*. From the fact that irradiation *in vitro*, with the dose employed, did not kill the tumor — as proved by Exp. II — he further concludes that the failure of the inoculation to take in the two surviving mice of Exp. IV must have been due to a protective effect from the roentgen dose given. It is undoubtedly wrong, however, to suppose that all the deaths may not very well be due to the irradiation. I have myself very often seen death result, in mice, from similar roentgen

doses over a limited area; and those deaths may just as well occur on the sixth day as on the ninth. That the two failures to take should furnish a sufficient basis for concluding to a protective effect of the rays is more than doubtful, indeed.

CONTAMIN (41), from his results with a very large experimental material, has found that it was possible by irradiation *in vitro* to kill the tumor, to reduce the proportion of takes, and to restrain the energy of growth. In connexion with one of his experiments he speaks of the secondary radiation of the lead, and believes that he was able to detect a not inconsiderable influence on the virulence of the tumor from that source. I have not found it possible, on the basis of such information as he gives in his treatise, to reduce his doses to any of the known units of measurement.

In 1912, WEDD and RUSS (240) published a paper on the effect produced by rays of various qualities on tumor tissue *in situ* and *in vitro*. They worked with a spark-length of from 6 to 10 cm., and the distance from the tube was 2 cm. When soft rays were called for, they did not use any filter; to obtain rays of medium hardness they filtered through 0.56 mm. of aluminum; to obtain rays of the hardest quality, through 1.12 mm. al. The strength of their primary current was 4 ampères, that of the secondary current from 0.35 to 0.8 milliampères. The irradiated layer of tumor tissue was 2 mm. thick; the temperature in the glass containing it, between 24 and 30°.

There were no takes after soft radiation for 30 minutes, nor after medium-hard radiation for 1 hour; but with the hardest rays the irradiation had to be kept up for 2 ½ hours in order to prevent takes. RUSS (198), WINTZ and RUMP (249) and others have since found further substantiation for the belief that soft rays have a stronger biological effect than hard ones.

In the paper just referred to, WEDD and RUSS also describe the result of two isolated experiments which they made with transplantation immediately after irradiation of the tumors *in situ*. Also in those experiments they found that the tumors were not killed in spite of irradiation with what would seem to be quite enormous doses of roentgen; namely, 4½ hours daily for three days in succession.

HILL, MORTON and WEATHERBEE (80) have made two series of tests with irradiation *in vitro*, implantation of the tissue thus irradiated, irradiation *in vitro* of the resulting tumor, implantation again of the latter, and so on. For the first series they used a Coolidge tube; spark-length, 8 inches; exposure, 2 minutes 36 seconds; and 14 generations were transplanted. In the first nine of these the percentage of takes was somewhat lower from the irradiated tumors

than for the non-irradiated ones, while in the tenth to fourteent no such difference was observed. In about one half of the cases th irradiated tumors appeared about one week later than the non irradiated ones, but after about five weeks' time they nevertheles were of practically the same size.

The second series of experiments was made with a spark-lengt of 1 inch; strength of current, 25 milliampères; distance, 8 inches exposure time, 20 minutes. In this series the tumor was only fol lowed through four generations, and the result showed a consider able reduction in the percentage of takes, and a lengthening of th period of latency, at least as regards the two first generations For the third and fourth generations the period of latency was al most the same. Nor with regard to these experiments am I abl to state the dosages in the terms of any known or generally em ployed unit of measurement; and that is the reason why the re sults arrived at by these writers have not found a place in the ac companying table. It seems as if there had been an inhibitory effec on the tumor tissue at first; and the first experiment furthe shows that any such inhibition apparently disappears after re peated irradiations.

In 1920, Wood and Prime (254, 255, 256) published an importan study on irradiation of tumors *in vitro*. They had used in thei experiments a rapidly growing large-celled sarcoma and a medullar mammary sarcoma, both from mice. The tumor tissue was placed i a damp chamber, covered with a piece of glass from 0.15 to 0.1 millimeter thick. Before being irradiated, it was cut into piece of as nearly as possible equal size, each piece weighing about milligram. The irradiation was done through 3 mm. of aluminum the spark-length was 21 cm.; the tension 25 kilovolt; the strengt of the current 5 milliampères. With this arrangement it took 1 minutes to give 1 HED. Only after 25 minutes' irradiation — tha is, after a little over 2 HED had been given — was there any lessen ing in the proportion of takes and rapidity of growth; and onl after irradiation for more than 70 minutes were there no mor takes. The carcinoma proved slightly more radiosensitive tha the sarcoma; thus, it only required 20 minutes' irradiation to re duce the activity of its growth and the frequency of takes, and 6 minutes to kill it. In order to find whether the effect was the same or different, on tumors in the living organism, a number of ani mals were irradiated for from 10 to 90 minutes under experimenta conditions precisely similar, and superficial fragments of thei tumors transplanted onto a fresh set of animals. In those condi tions it was found that it took 20 minutes' irradiation to produc

an inhibitory effect on the growth of a sarcoma, and 60 minutes to prevent it absolutely from taking when transplanted; while the carcinoma had to be irradiated for 70 minutes before it ceased to take.

It, consequently, took:

about 6 HED to kill the sarcoma *in vitro*;
» 5 » » » » » *in situ*;
» 4 » » » » carcinoma *in vitro*;
» 6 » » » » » *in situ*.

For the sake of addititional control, fragments of the tumors that had been roentgen-rayed *in vitro* were planted out in tissue cultures, and at the same time fresh pieces of tumor tissue and embryonic mouse kidney were irradiated, likewise in tissue culture. It took:

5 HED to kill carcinoma cells in culture;
over 4 » » » sarcoma » » » ;
4 » » » normal embryonic kidney tissue in culture.

Keysser (98, 99) roentgen-rayed, *in situ* on dead mice, tumors of the size of hazel nuts, and found that they were not killed even by 192 per cent. of the dose which both Seitz and Wintz (216, 217), and Kroenig and Friedrich (110), call the carcinoma dose. The tumor in question was a very malignant spontaneous carcinosarcoma from the jaw of a mouse. The irradiation was done with a Coolidge apparatus: strength of current, 2 milliampères; tension, 160 kilovolt; tube-to-skin distance, 23 cm.; filter, 0.5 mm. zinc + 1 mm. aluminum. With this arrangement it took 45 minutes to give 1 HED.

Wetzel (247) has roentgen-rayed a giant-cell mouse sarcoma which gave 100 per cent. of takes when transplanted. The tumors, at the time of irradiation, were from 0.96 to 1.16 centimeter in diameter. He irradiated for 10, 12 ½ and 15 hours, using a Veifa »Intensive Reform» apparatus with Coolidge tube, and filtered through 0.5 mm. cu. + 1 mm. al. The distance was 80 cm.; strength of current, 2 milliampères; tension, 160 kilovolts. The tumors were irradiated *in situ* and transplanted at various times, up to 17 days after irradiation. Even with those doses — which correspond to 5 Sab., or less than 2 HED — it was found impossible to prevent takes from the transplanted tumor. Wetzel believes that he has noticed a stronger growth of the tumors after irradiation for 8 hours at a distance of 60 cm. through 1 mm. cu. + 1 mm. al., and

also that the frequency of takes after inhibiting doses becomes greater as the distance in time, from the irradiation, increases.

Opitz (173) asserts — though without stating the source of his information — that carcinoma cells may be irradiated *in vitro* with as much as ten times the »carcinoma dose» without losing any of their vitality.

Murphy, Maisin and Sturm (148), in their work on »Local Resistance to Spontaneous Mouse Cancer induced by X-rays» state that a tumor which has been subjected *in vitro* to 1 HED grows just as well after being implanted in the skin as one that has not been roentgen-rayed. To give 1 HED unfiltered they used 2½ minutes, with a spark-length of 3 inches; skin-to-focus distance 6 inches; strength of current, 10 milliampères.

Russ (240, 199) — using the same technic as for his work in collaboration with Wedd — showed that irradiation with soft rays for 42 minutes reduced the proportion of takes from Jensen's rat-sarcoma by 50 per cent.; and that irradiation for 56 minutes prevented takes altogether. He also determined the lethal dose for hard rays, and set up the following table, which shows the relative effect of the two qualities of rays:

	Lethal Dose for Tumors	Partial Epilation	Vesiculation
Soft Rays (45—30 ÅU)	56 minutes	21 minutes	38 minutes
Hard » (168 ÅU)	146 »	126 »	228 »

The difference between the lethal dose for a sarcoma of rapid growth and the one needed to kill a slow-growing carcinoma he found to be 20 per cent., at most. For comparison he states that a series of widely different malignant human tumors did not give any takes when homologously transplanted after exposure to a roentgen dose twice as strong as the lethal dose for Jensen's rat-sarcoma.

In the last part of his paper, Russ deals with the question of the effect of radium on the tumor *in vitro*. He establishes the lethal dose, and shows that the tumor tissue becomes less resistent both to roentgen and radium after it has been subjected to repeated sub-lethal doses of the latter. That the same could be the case with malignant human tumors had already been shown ten years before, by Nogier and Regaud (171). This may perhaps be due to the fact that the radiosensitivity of the cells is highest in the stage of division, and that the number of mitoses can be reduced by irradiation with roentgen, as shown by Béclère (21, 22), Tribondeau

and Bergonié (232), Regaud (189, 190, 191, 192), Markovits (132), Mallet (131) and Holthusen (83, 84).

Russ, in collaboration with Scott (201), showed that the lethal dose for five different rat- and mouse tumors did not vary more than 30 per cent., at most, from the dose required for killing Jensen's rat-sarcoma. A dose five times as strong as the latter was insufficient to restrain the growth of Rous's sarcoma, and a dose three times as strong had no influence on the filtrate of that tumor. His experiments did not extend to doses of greater strength than this.

Nakahara (160) found that Bashford's carcinoma no. 63 was uninfluenced by 1 HED. I have myself shown (109) that the same dose, unfiltered, did not have any demonstrable effect on squamous-cell tar carcinomata from a mouse.

Kok and Vorlaender (102, 104, 105, 106, 238), who worked with a very malignant carcinoma from Ehrlich's Institute, found that the latter, *in vitro*, was not killed by 1,500 *e*. They used partly a »symmetry» apparatus from Reiniger, Gebbert and Schall, with Coolidge tube; partly a »radiosilex» apparatus with Liljenfeldt tube. The latter probably worked with a tension of 80 kilovolts and a current of 8 milliampères. The »symmetry» apparatus was worked with a parallel point-to-plate spark-length of 35 cm.; strength of current, 2.5 milliampères; focus distance, 20 cm.; filter, 0.5 cu. It took from 12 to 15 seconds to give 1 *e*. It is difficult to say exactly how much 1500 of those represent in terms of HED; but it would probably equal about 9 HED. Kok and Vorleander also roentgen-rayed the tumor *in situ*, and found that it could be killed with slightly less than the epilatory dose, which, under the given conditions, was equal to about 250 *e*. They got just as good results from all-over exposure of the animals to 25 *e*., as from local irradiation of the tumor with 250 *e*. When the animals were roentgen-rayed before being inoculated, the best results were obtained by local irradiation with 275 *e*. and general irradiation with 25 *e*. I shall come back later to their experiments with implantation *after* local irradiation.

With regard to the effects of the general irradiation, and the optimum of dosage for irradiation of tumors *in situ*, I have no experiences of my own, on the basis of which I might venture to contribute to the subject.

Of recent years, a number of experiments have been made with irradiation of tumors in tissue culture. Of those undertaken, in 1920, by Wood and Prime (254) I have already spoken. Already three years before, Prime (186) had studied the action of radium

on tissue in cultures, and believed that he had noticed a stimulat ing effect from small doses.

KIMURA (100) has worked with one carcinoma and one sarcoma and has shown that for both forms of tumor it requires the sam dose to prevent takes after transplantation. He thought that h had noticed an increase in the number of mitotic figures after sma doses of roentgen.

Though it is very difficult from the information given by th various writers to make a comparison of the quantities of roentge used, I nevertheless believe it may safely be taken as established tha *the dose required to kill a tumor tissue by irradiation either outside th organism or in its position in a living or dead animal whence it i removed immediately afterwards, far exceeds the largest single dos that it is possible to employ therapeutically.*

Expressed in units of HED, the results arrived at by the variou experimenters would seem to figure out as follows:

MEISEN WESTERGAARD kills tumors with about 1 ¼ HED o unfiltered roentgen;

CLUNET is unable to kill a malignant sarcoma with about HED of unfiltered roentgen;

WEDD and RUSS kill tumors with about 5 HED through from ½ to 1 mm. al.;

WOOD and PRIME kill tumors with about 4 to 6 HED through 3 mm. al.;

KEYSSER is unable to kill tumors with about 2 HED through ½ mm. zinc + 1 mm. al.;

WETZEL is unable to kill tumors with about 1 HED through ½ mm. cu. + 1 mm. al.;

OPITZ is unable to kill tumors with about 10 HED (filter no stated);

RUSS kills three different tumors with about 5 HED through 3 mm. al.;

KOK and VORLAENDER are unable to kill tumors with about HED through ½ mm. cu. + 1 mm. al.;

MURPHY, MAISIN and STURM fail to make any impression on malignant tumors with 1 HED of unfiltered roentgen;

NAKAHARA makes no impression on Bashford's carcinoma no. 63 with 1 HED of unfiltered roentgen;

KREBS makes no impression on a squamous-cell carcinoma with 1 HED of unfiltered roentgen.

WEDD and RUSS, WOOD and PRIME, and RUSS — all of whom worked with a very large material — arrived at about the same

result for the lethal dose. The experience of KOK and VORLAENDER loses a great deal of its importance by the fact that they only used 3 animals in their experiments. OPITZ's statement I have not been able to verify, as he does not give his source. That the results arrived at by MEISEN WESTERGAARD and CLUNET differ so widely, both among themselves and from those of the writers first named, is less easy to understand. The most likely explanation is probably to be found in the as yet incomplete development of the technic of measuring at the time when they made their experiments.

A great many things seem to indicate that the lethal dose for a number of different tumors, and for a few normal tissues, is more or less the same, and may be taken to lie between 4 and 6 HED.

MY TECHNIC OF IRRADIATION

The irradiation was done by means of a »symmetry» apparatus from REINIGER, GEBBERT and SCHALL. The voltmeter showed 142 K.V., measured on the primary current. The strength of current was 2.8 milliampères. In most of the experiments the filter was 2 mm. of aluminum; in a few, 5 mm. al.; in one experiment unfiltered rays were used. It took 3½ minutes to give 1 Sab. & Noiré through 2 mm. al., and the clinical experience showed that 3 S.-N. through 2 mm. al. was = 1 HED. By 1 HED through 2 mm. al. on a field of 10 × 10 cm. is understood the quantity of roentgen that given in one dose will produce in about two weeks a distinct erythema with resulting pigmentation.

In all the experiments the skin-to-tube distance was 23 cm. To kill a mouse by general roentgen irradiation requires about 3 S.-N. through 2 mm. al.; twice that dose locally, on a field 2 × 3 cm. in diameter, produces epilation. The same Coolidge tube was used in all the experiments.

MY OWN EXPERIMENTS CONCERNING THE ROENTGEN EFFECT ON TUMOR TISSUE IN VITRO AND IN SITU, AND THE LETHAL DOSE FOR SUCH TISSUE

In my experiments for the above purpose I used in all 835 mice, of which 300 were control animals. Each series comprised from 10 to 40 animals; one particular series as much as 70 — all besides the controls. The experiments extended over a period of 18 months. The purpose of the first ones was to show what happens to the tumor tissue — *in vitro* or *in situ* — when subjected to various doses of roentgen and transplanted immediately afterwards.

I have not concerned myself with the histological changes that take place in the tissue as a result of the irradiation, but have confined myself to the investigation of the following four features, as purely macroscopical measures for the vitality of the tumor:

1) Proportion of takes;
2) Duration of the animal's life after transplantation;
3) Period of latency;
4) Rate of growth.

In the case of the carcinoma, the percentage of takes (as already mentioned on p. 19) varied from about 40 per cent. to about 60. The variations within a limited time were rather slight, however; and it rather seems as if there was a steady, gradual rise in the percentage throughout the years; while, in the case of the sarcomata, the proportion of takes — except for the very earliest generations — remained fairly constant, at about 95 per cent. With regard to the carcinoma, those variations in the proportion of takes, and in the duration of the life of the animals, make it necessary to be careful, and not to draw conclusions from the more or less deviating result of any separate experiment, but rather to consider and judge from the aggregate of them, as a whole. In the following tables, the takes are given either in the form of a fraction, in which the numerator is the number of takes, and the denominator the number of animals still alive 48 hours after the operation; or else

by a figure indicating the number out of 10 animals, in which the transplantation was successful.

As regards the sarcoma, the rate of growth was fairly even. In ten days the tumor would be about the size of a hazel-nut; after about twenty-five days as large as a plum. Of course, there might be slight variations; but from the tracings made right along one got the distinct impression of a fairly uniform, even growth. A further proof of this is to be seen in the fact that also the life time of the control animals varied very little within each separate experiment.

In a general way, the same was the case as regards the carcinoma; though, here, some more considerable deviations were noted from time to time, which also were reflected in the duration of life of the control animals.

By »duration of life» I mean the time that elapses from the inoculation to the spontaneous death of the animal. It should be remembered, however, that in the case of some of the animals death may possibly occur as the result of some intercurrent disease. The figures pertaining to this point should therefore be accepted with some little reservation.

In the carcinoma experiments, the duration of life after inoculation has been figured out for 71 control animals. That the number is not larger is due to the fact that I had not fully realised, at first, the importance of this factor of »average life», and that I had therefore simply ascertained whether there were any takes or not, whereupon the animals were used for other experiments. The same was the case with the mice in the sarcoma experiments. Still, of those, the average life was figured out for 160 animals. With regard to the carcinoma-mice it was found that the duration of life varied enormously, the two outside figures being 29 and 228 days, respectively. Those were exceptions, however. The average was 64.6 days. For the sarcoma-mice the variations were much less; the outside figures being 12 days and 77 days, respectively. Even so, the latter case was distinctly an exception. The average for this category was 30.6 days.

By »period of latency» I mean the time that lapses from the day of inoculation until the appearance of a palpable tumor. To give the length of this period as exactly as the percentage of takes and the duration of life is, of course, impossible; in the first place because one could not well manage to palpate the animals every day; in the second place because it is impossible to decide by palpation alone whether the swelling is an incipient tumor or merely an inflammatory infiltration. There is hardly any doubt but that

there may sometimes appear, after the inoculation, a swelling about the size of half a pea, which must be regarded in the nature of an inflammatory reaction. I have repeatedly, after implantation of a strongly roentgen-rayed tumor tissue, observed, in from ten to twenty days' time, the development of a small lump at the point where the implantation took place, which would eventually disappear completely, and only later — sometimes after several months — be replaced by a permanent, true tumor.

On the other hand, if the intumescence is a steadily growing one, it is, of course, just as impossible to judge by palpation alone whether the tissue is inflammatory or neoplastic in character. As the »period of latency» is thus more or less a matter of estimate, I have not attempted to state it in any definite figures of time. The experiments have shown that any lengthening of it only occurs in connexion with large doses, and that it increases proportionally with the latter.

Whether the tumor cells are at rest for a shorter or longer time during this »period of latency», or whether the mitosis takes place more slowly to begin with, after large doses of roentgen, is more than I would venture to decide. It is also possible that the rate at which the division occurs is the same all the time, and that any variation in the rate of growth during the first period following the inoculation only depends on the larger or lesser number of viable cells present. The variations that may be due to difference in the size of the implanted tissue-fragments would certainly be so small as to be wholly negligible; seeing that, as already stated, particular care was taken to have all the fragments cut to a very uniform size. I admit my inability to account for the process taking place in the tumor tissue during the period of latency; and I am thus using that term merely as the expression for an inhibition, the nature of which I am unable to explain.

Already Perthes (180) called attention to the fact that the roentgen- and radium rays can reduce the number of cell-divisions and prolong the period of mitosis. This has since been confirmed by experiments in tissue culture; and if it is permissible — as I am inclined to believe — to conclude from the results of those experiments, there is a certain probability that the mitoses, during the period of latency, have either become fewer in number or ceased altogether. It has been shown by Kimura (100) and Scholtz (210), on malignant tumors, and by Strangeways and Oakley (226) on cultures of the chorioid from chicken embryos, that irradiation with sublethal doses makes the mitosis abnormal and scanty; and Scholtz (210) has furthermore shown that the whole process of

cell-division can be made to cease for a while, and then commence again.

It is thus only in theory that it is possible to make a distinction between rate of growth and period of latency. I have therefore not attempted to establish them separately, but have confined myself to recording, diagrammatically, the size of the tumors at various times after inoculation. Those tracings give a good idea of that which the two terms are meant to express — namely, the »energy» of the tumor's growth.

The following experiments fall into four parts; the doses having been tried both in relation to carcinoma and to sarcoma, *in situ* as well as *in vitro*.

I chose to test the doses: 3, 6, 9, 12, 15, 18 and 21 S.-N., which correspond more or less to: 1, 2, 3, 4, 5, 6 and 7 HED. I used a filter of 2 mm. aluminum, with a skin-to-focus distance of 23 cm. The filter was chosen with a view to making the irradiation time as short as possible for the sake of the animals, at the same time as it answered my wish of using one that would be serviceable also in cancer therapy. For irradiation *in vivo*, the area immediately surrounding the tumor was left unprotected, while the rest of the animal was covered by a sheet of lead 2 mm. thick. With this arrangement it took 3 ½ minutes to give 1 S.-N.; and it was found that under the given conditions, and with the apparatus I used, 3 S.-N. was equal to 1 HED.

The experiments extended over a rather long period; and as it is well known that animals of this species differ not a little as regards susceptibility to transplanted tumors, it was natural to expect some variation in the results, depending on such individual conditions as age, development, pregnancy, lactation, feeding, strain, health, and probably a number of other, unknown factors. All those that were known, or could be foreseen, I tried to eliminate by always choosing for experiment and control animals of the same weight and sex, belonging to the same lot, and who, up to the day of the experiment, had been living under practically the same conditions. Animals found to be pregnant were eliminated. No animal weighing over 20 grams was used. By not exceeding that weight-limit it may, namely, be taken for fairly certain that two mice that weigh the same number of grams are also of nearly the same age. The tissue used for transplantation I took from tumors that were all of the same size and age. It is evident that under conditions otherwise equal the most reliable results will be obtained from experiments comprising a great number of animals and carried out within

the shortest possible space of time. The time and assistance over which I have been able to dispose has set some limits, however, to the amount of work that I could accomplish each day; I have therefore mostly had to confine myself to experiments involving relatively few animals each. It is especially for a comparison of the average duration of life that it is difficult to obtain the proper basis unless pieces from the same tumor are used, and unless the animals on which the inoculation is made constitute a strictly homologous material.

I made in one day one experiment comprising 70 animals as alike as it is possible to obtain them. As it might be expected, the result shows the effect of the rays on the tumor more clearly than it would be possible if the same experiment was done piecemeal, extending over a longer time, on a less uniform material, and using tumor tissue from several animals.

For the experiment here alluded to, the tissue was taken from mouse no. 1750, which had two tumors. The latter were cut into pieces each as large as half a pea, and the pieces were placed in small Petri dishes containing a little normal saline solution, a piece from each tumor in each of the dishes. During the irradiation the dishes were covered with a thin layer of sterilised paper, and over this again a thick layer of cotton in order to protect the tissue from the heat from the tube. This, as all the rest of my experiments, was done by room temperature; and it was controlled that the latter, in the immediate neighborhood of the dishes, never exceeded 20° C. Gradually, as the various doses had been given, the dishes were removed, and the pieces of tissue cut into still smaller fragments, each about 1 millimeter thick. These fragments were then implanted subcutaneously in the axillæ of the animals, in the manner already described; every animal getting a piece from each of the tumors. The implantation was done on batches of ten mice at the time. The whole operation extended over 8 hours; and in order to be sure that any change that might possibly occur in the tumor tissue during that time would not alter the results, the control animals were inoculated last. There were 20 of those control animals. Two of them died under the narcosis, the others lived. The results are seen in Fig. I and Table I.

Fig. 1.

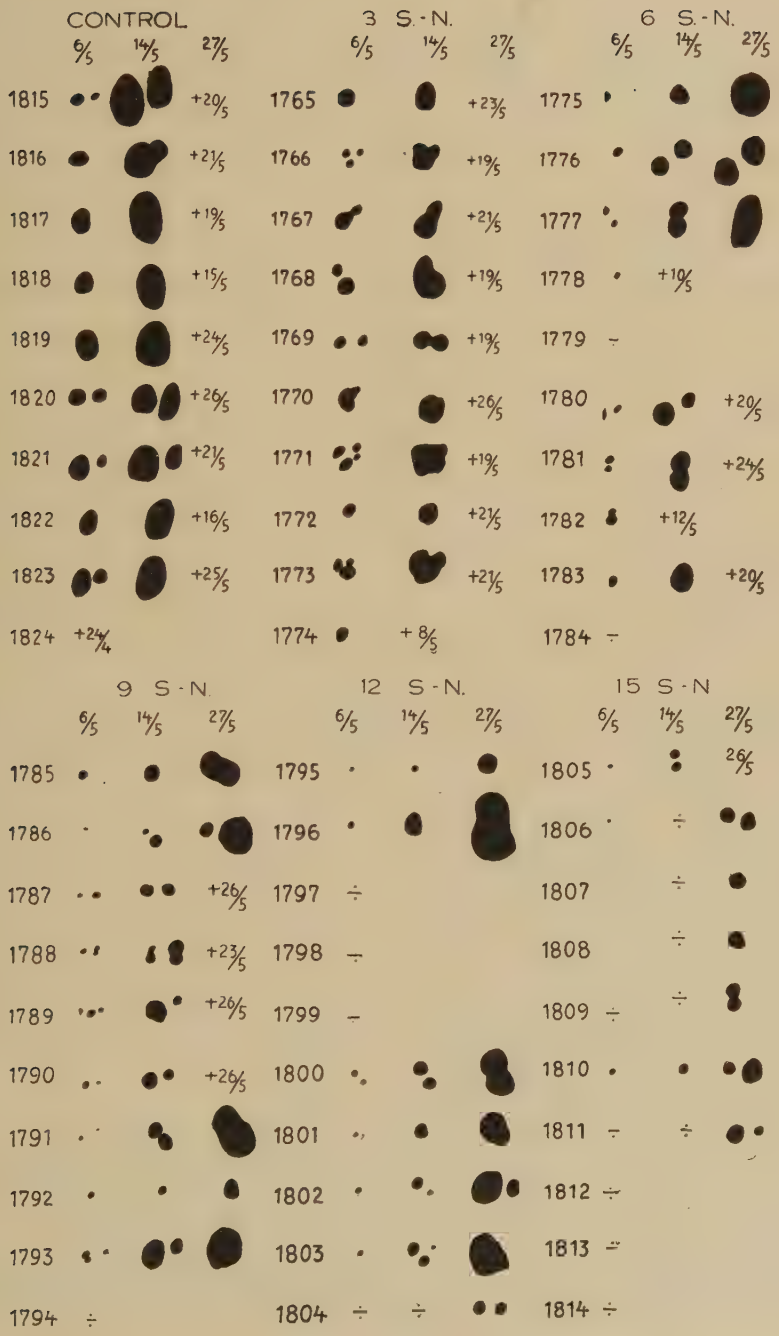

CONTROL
6/5 14/5 27/5
1815 +20/5
1816 +21/5
1817 +19/5
1818 +15/5
1819 +24/5
1820 +26/5
1821 +21/5
1822 +16/5
1823 +25/5
1824 +24/4
3 S.-N.
6/5 14/5 27/5
1765 +23/5
1766 +19/5
1767 +21/5
1768 +19/5
1769 +19/5
1770 +26/5
1771 +19/5
1772 +21/5
1773 +21/5
1774 + 8/5
6 S.-N.
6/5 14/5 27/5
1775
1776
1777
1778 +10/5
1779 ÷
1780 +20/5
1781 +24/5
1782 +12/5
1783 +20/5
1784 ÷
9 S.-N.
6/5 14/5 27/5
1785
1786
1787 +26/5
1788 +23/5
1789 +26/5
1790 +26/5
1791
1792
1793
1794 ÷
12 S.-N.
6/5 14/5 27/5
1795
1796
1797 ÷
1798 –
1799 –
1800
1801
1802
1803
1804 ÷ ÷
15 S.-N.
6/5 14/5 27/5
1805 26/5
1806 ÷
1807 ÷
1808 ÷
1809 ÷ ÷
1810
1811 ÷ ÷
1812 ÷
1813 ÷
1814 ÷

Table I

Average Duration of Life after:	3 S.-N.	6 S.-N.	9 S.-N.	12 S.-N.	15 S.-N.	*Controls*
	days	days	days	days	days	days
	29	36	38	53	32	27 + T (+ M)
	25	41	38	34	51	27 out
	27	38	32	— T	53	25 31
	25	16	29	— T	51 (+ M)	21 + T
	25	— T	32	— T	46	30 out
	32	26	32	34	38	32 30
	25	30	34	35	41	27 27 (+ M)
	27	18	41	39	— T	22 26
	27	26	34	41	— T	31 20
	14	— T	— T	39	— T	+ T 25
	256 : 10	231 : 8	310 : 9	275 : 7	312 : 7	401 : 15
	=25.6 days	=28.9 days	=34.4 days	=39.3 days	=44.6 days	= 26.7 days
No. of takes per group of 10 animals:	10	8	9	7	7	10

Obs. — T = no tumor; + T = tumor, but animal killed in order that the growth might be used for re-transplantation; + M = macroscopic metastasis to the lungs.

From the above it will be seen that doses of less than 9 S.-N. do not apparently have any influence on the percentage of takes or on the duration of either the period of latency or the life of the animals, while the rate of growth is noticeably diminished already after 6 S.-N. From 9 S.-N. upward, there is a distinct effect on both the average duration of life, the period of latency and the rate of growth, while the proportion of takes remains the same; and it is only from 12 S.-N. upward that the latter too is seen to become noticeably less.

In this manner, the whole series of experiments, as indicated on p. 45, was carried out with doses up to, and including, 21 S.-N., both for carcinoma and sarcoma *in situ* as well as *in vitro*. For irradiation *in situ* the animals were pegged out under ether during the treatment, and the grafting done, immediately after they had been roentgen-rayed, with fragments from the superficial layers of the tumors. All the results point in the same direction as those of the first experiment. As all the measurements which I am using to define the degree of the roentgen effect are expressions of animal vitality in one form or another, it is unavoidable that there should be some variations within the single experiment, seeing that each

comprises so relatively small a number of animals. The best judgment will therefore be had by considering the aggregate results, as shown in the table below.

The tables giving the results of the individual experiments will be found at the end of this volume. In all, thirthy-seven experiments were made. Some of those in which the roentgen dose was so large as to produce a very marked effect were repeated — one of them twice.

Table showing the aggregate results from irradiation of carcinoma and sarcoma in situ and in vitro

	Carcinoma						Sarcoma					
Dose S.-N.	in situ	No. of takes in 10	Average of life, days	in vitro	No. of takes in 10	Average of life, days	in situ	No. of takes in 10	Average of life, days	in vitro	No. of takes in 10	Average of life, days
3	9/10	9	61	7/19	3.7	61	10/10	10	43	6/10 10/10	8	33
6	7/10	7	76	6/15	4	77	10/10	10	43	9/10 8/10	8.5	42
9	9/20	4.5	87	6/10	6	75	5/10	5	63	7/10 9/10 20/24	8.2	47
12	1/19 8/20	2.3	97	7/10 10/20	5.7	93	4/10	4	80	0/10 7/10	3.5	39
15	1/20	0.5	146	1/15 5/20	1.7	95	5/10	5	59	7/10 0/10	3.5	45
18	3/20	1.5	77	3/20	1.5	143	2/19	1.1	112	1/10	1	51
21	0/20	0	—	0/19	0	—	0/20	0	—	0/10	0	—
Control:	24/60	3/10	6/10	6/10	4/10	16/20	7/9	10/20	8/10	9/10 18/20	7/10 19/20	6/6 10/10 10/10 17/17	10/10	18/18 19/20

We thus have, for

		No. inoculated	No. of takes	Percentage
CARCINOMA (*in situ* and *in vitro*)	Controls	159	84	= 52.8
	3—6—9 S.-N.	84	44	= 52.4
	12—15—18 »	164	39	= 23.8
SARCOMA (*in situ* and *in vitro*)	Controls	151	143	= 94.7
	3—6—9 S.-N.	114	94	= 82.5
	12—15—18 »	89	26	= 29.2

The sum of the experiments proves that the lethal dose f these two tumors, whether *in situ* or *in vitro*, is the same; namel 21 S.-N. We also notice, just as in the large experiment (Tab I), that, in the case of the carcinoma, doses of less than 9 S.-N hardly alter the proportion of takes — the percentage bei 52.8 for the controls and 52.4 for the roentgen-rayed animal while, after irradiation with 12, 15 or 18 S.-N. the percentag for the latter sinks to 23.8. As regards the sarcoma, there seem to be a slight drop in the percentage of takes after 3, 6 an 9 S.-N.; namely from 94.7 to 82.5; while, after 12, 15 and 18 S.-N there is a similar considerable fall as in carcinoma; namely, 29.2 per cent.

For carcinoma *in situ*, the proportion of takes descends evenl with increased strength of irradiation, from 90 to 0 per cent.; an at the same time, the average duration of life — which, for th control animals, was 64 days — increases by even degrees, from 6 days after 3 S.-N. to 146 days after 15 S.-N. Why the duratio of life after 18 S.-N. was only 77 days, I cannot explain, unle it may possibly have been due to some intercurrent disease in th animals, or to other causes not directly related to the developme of the tumor. For carcinoma irradiated *in vitro* there is likewi a drop in the percentage of takes, but not as evenly gradual a elsewhere; while the increase in duration of life is almost the sam as after irradiation *in situ;* namely, from 61 days after 3 S.-N to 143 days after 18 S.-N.

For sarcoma, the decrease in percentage of takes is even an gradual, alike for the tumor irradiated *in situ* and for the one i radiated *in vitro;* while the average duration of life for the anima of the former category increased from 43 to 112 days, for those o the latter category from 33 to 51 days.

Whenever macroscopically demonstrable tumors were foun in the organism, these were examined under the microscope; wit the result that metastasis of the carcinoma was found in 7 per cen of the controls, and in 14 per cent. of the animals inoculated wit irradiated tissue. Metastasis of the sarcoma occurred in a differe proportion; namely, in 16 per cent. of the control-animals, and i 10 per cent. of the others. In this calculation no account is take of the animals in which the transplantation failed to take. Fo this contrast between carcinoma and sarcoma, in the matter o metastasis, I am unable to find any explanation. It is possible tha the figures might have turned out differently if I had extende the examination to a microscopic search for metastasis also in cas where there was no macroscopically visible tumor.

As already mentioned on p. 41, it seems that the lethal dose for a number of different malignant tumors lies between 4 HED and 5 HED, and that an inhibition may occur after 2 HED. That some experimenters should have found it to lie at the former dose, and others at the latter, need not imply any really important difference, when one considers how varied — and often inexact — have been the methods of measurement employed.

The conclusions which I believe myself justified in drawing from the results of my own experiments I would state as follows:

I. — *An absolute, directly lethal effect is produced on the cancer cells only by doses of more than* 6 HED. *In the case of the sarcoma and the carcinoma with which I worked, the effect of that dose was the same for both types, and that irrespectively of whether the tumors were roentgen-rayed in situ or in vitro.*

II. — *Smaller doses may restrain the energy of the tumor's growth; this inhibitory effect showing itself partly in a lengthening of its period of latency, partly in a reduction of its rate of growth.*

THE INFLUENCE OF GENERAL ROENTGEN IRRADIATIO ON THE DEGREE OF RESISTANCE TO MALIGNANT TUMOR

In fighting cancer, as in fighting bacterial diseases, two roac are open; namely, the direct one of attempting to destroy the patho logic agent — in one case the bacilli, in the other the neoplast cells; and the indirect one, which consists in attempting to stimu late the natural defenses of the organism itself to resist the in vading enemy. The fact that there seems to be certain points of sim ilarity between the organism's manner of reacting to infectio and to neoplastic growth makes it natural to ascribe that reactio to the same cause in both cases; namely, to the formation of anti bodies. The idea is equally justified whether one believes tha tumors are due to a microorganism or not. In the former case thos antibodies might be cytolytic ones, in the latter bacterial.

The first part of these experimental investigations has dea with the effect of that now commonly used direct cancer-killin agent, the roentgen ray, upon the vitality of the cancer cell I shall in the following pages proceed to discuss the more indirec agents.

Most of the experiments that have been made with a view t studying the organism's power to destroy the neoplastic cell hav been made with implanted tumors, and it must be remembered tha it is very doubtful whether the reaction of the organism towar those is the same as toward the spontaneous growth. That diffe ences in that respect exist is unquestionable. We need only men tion such circumstances as the fact that most of the spontaneou tumors, by far, are found in females and old mice, while the implan ted tumor takes equally readily in mice of both sexes, and decidedl more readily in young specimens than in old ones. Another indica tion of the same difference is found in the fact that artificial a well as spontaneous tumors may occur in mice that are absolutel resistant to implanted cancer.

The experimentation, then, has followed the same lines wit regard to tumors as with regard to bacterial diseases; that is, bot active and passive immunisation have been tried. The earlie

ttempts to produce the former were made by means of specifics, uch as the injection of tumor tissue; later, non-specific means were ried, such as numerous normal tissues, dry heat, roentgen, etc.

It is as yet impossible to define the nature of the defensive owers possessed by the organism. If the latter is resistant, a tumor mplanted in it will either fail to take, or else it will be resorbed fter it has grown to a certain size. It may be that some organsms are lacking more or less completely in certain, as yet unecognised substances indispensable to the thriving of the neolastic tissue; or it may be that some actively inhibitory endogenous orce asserts itself, either released as a reaction against the growth f the malignant tissue, or perhaps, in other cases, already present n a measure sufficient to prevent the tumor altogether from estabishing itself. If the latter is the case, the next question is: to what articular character of the organism are those defensive powers elated? — where are they developed, and where are they found? — what is their nature, and how do they express themselves? — an they be increased, if not sufficiently strong? — can they be reated where they do not already exist, and if so, how?

Those are but some of the innumerable questions that present hemselves immediately one begins to study the cancer problem. Most of them are impossible to answer. Only so much can be said, hat, up to the present, none of the known antibodies have been ound in that connexion; neither the coagulating principles (aggluinins, precipitins, etc.) not the dissolving substances with comlement-fixing properties (bacteriolysins, hemolysins, cytolysins, tc.). The fact is that the immunity to tumors cannot be classed nder the heading of any form of immunity known to us at present.

Numerous experiments have been made with immunisation gainst tumors. Also in that field, C. O. Jensen (88, 89) has been pioneer. Already in 1902 he published the successful results of is attempts to produce passive immunisation of mice by means f a serum from rabbits inoculated with mouse-tumor tissue. Nearly ll the attempts at passive immunisation have given a negative esult. Only a few experimenters, like Clowes (40), Michaelis 133), Sticker (222) and Lewin (118, 120), think that they have btained some good effects from that proceeding. Better and more ositive results have been obtained by active immunisation. We ave said that the earliest attempts in that direction were made vith injection of neoplastic tissue, especially with that of slightly irulent malignant tumors. Thus, Ehrlich (51), for instance, used emorrhagic adenocarcinomata from mice. Later it was found that ust as good results were obtained when normal tissue was used;

and this led to the idea — which SCHOENE (213, 214) was the firs to suggest, in 1906 — that the immunity to malignant tumors not a specific immunity against cancer cells, but a non-specifi immunity against foreign tissue cells in general, and that the latte is due to the same factors that prevent a transplanted normal tissu from establishing itself on the new organism. Most of the writer are inclined to think that the inhibitory forces are related to th cells, and not to the humors of the body. Still, the most recen experiments made, by ALBERT FISCHER (61), with Rous's sarcom would seem to indicate that — in the case of that type of tumo at least — there are two principles at work: one which produce immunity against the taking of transplanted tissue in genera and another, anticontagious one, which he believes to reside in th serum. Only where both those are present does absolute immunit against Rous's sarcoma exist.

FISCHER thinks that the immunity against tissue cells may b due to incapacity, on the part of the transplanted cells, to mak use of certain strictly specific — solidistic, cytoplasmic — prin ciples of growth assumed to exist in the cell-plasma. He thus re verts, at least partly, to the atreptic theory of Ehrlich's.

Among investigators who have attempted to immunise agains cancer by means of tumor-tissue injection may be mentioned C. C JENSEN (89, 90), EHRLICH (51), APOLANT (8, 11), BASHFORD (17, 18) BRIDRÉ (28, 29), CLOWES (40), CLUNET (35, 36), HAALAND (69, 18) LEWIN (117), MURRAY (17, 18), GIERKE (18), CRAMER (17), RUSSEL (204), DA FANO (53) and WOGLOM (250). HIRSCHFELD (81) has produ ced immunity by crushing the already developed tumor. He believe that in this manner vaccine is formed *in loco*. The first attempt at immunisation by injection of normal tissue were made by SCHOEN (214), who used mouse embryos, liver, spleen and the blood o mice for the purpose; and by LEWIN (118), BASHFORD, MURRA and CRAMER (17), who used blood. LEWIN and MEIDNER (121 used spleen tissue from tumor-bearing animals. APOLANT an MARKS (12) used spleen tissue from healthy animals, and also trie to render animals already suffering from tumors immune by in oculating them with tissue from their own spleen. BRIDRÉ (28) LEWIN (118) and APOLANT (11) used blood, liver, spleen and em bryonic tissue; WOGLOM (250) fetal skin; HIGUCHI (79) blood, pla centa, spleen and mammary tissue; TSURUMI (233) embryoni skin; ITAMI (86) the lenses, muscles and lymph glands of rats; D FANO (53) blood and embryonic skin. Later, ITAMI (87) repeate all his experiments, trying with nearly all the organs and tissues o the organism, as well as with whole embryos, placenta, lymphocytes

leucocytes and washed red blood corpuscles. ITAMI had imagined that the immunising power of the tissues might possibly be directly proportional to their richness in nuclei; but the error of this was proven by the fact that muscular tissue, for instance, had no effect, while, on the other hand, the washed red blood corpuscles may produce a certain degree of immunity. According to JENSEN (88), MOTTRAM and RUSS (140), the mixture of serum or plasma from immune animals with the tumor tissue, before the latter is injected, does not seem to affect the viability of the tumor. Another valuable Danish contribution to experimentation along this line is furnished by FIBIGER and POUL MØLLER (56), who have shown that injection of the skin of mouse embryos may reduce the frequency of metastasis formation in mice affected with tar-cancer.

Some writers have experimented to ascertain in what manner the injection of roentgen-rayed tumor tissue affected the degree of resistance. Opinion is somewhat divided. CONTAMIN (41) obtained an increased resistance to cancer by injecting tumor tissue that had been feebly roentgen-rayed, but got no effect from similar tissue that had been exposed to large — he does not specify *how* large — doses of roentgen. CHAMBERS, SCOTT and RUSS (39) believe that they obtained a higher degree of immunity by injecting tissue that had been killed by the rays, than from tissue that had been given only non-lethal doses. Some experiments with radium on animals with two tumors showed that if one of those tumors was subjected to a lethal dose the other one would disappear as well; but if only one third of the lethal dose was given, a certain reduction of the radiated tumor would result, but there would be no effect on the other at all. Some good results in the way of immunisation with roentgenkilled tissue are stated by KELLOCK, CHAMBERS and RUSS (97) to have been obtained by them when they inoculated patients suffering from cancer of the breast with tissue from their own tumors subjected to a roentgen dose twice as large as the one required to kill Jensen's rat-sarcoma. This is in absolute contrast to the experiences of WOOD and PRIGOSEN (253), whose experiments — with the enormous material of not less than 30,000 mice — show that injection of irradiated tumor tissue, whether in large or small doses does not produce the slightest immunity. Nor have NATHER (169), LEWIN (118, 119) or CLOWES (40) been any more successful in their attempts to produce immunity by the injection of dead tissue, no matter whether the latter had been killed by means of chloroform, autolysis or heating.

The conclusion to which we must come on the basis of all the experiments and results here enumerated is as follows: By the

injection of a great variety of tissues, healthy as well as pathc logic, and also of blood, it is possible to stimulate the power c the organism to resist malignant, implanted tumors. Whethe the same effect can be obtained by the injection of dead tissue is very doubtful. Antibodies in the serum do not seem to hav anything to do with this immunity; nor has it been possible t demonstrate with any degree of certainty the presence of suc restrictive substances anywhere else in this connexion.

We know practically nothing about the nature of the force thus mobilised; and if we wish to find out more about them, th only possible way at present is to study the manner in which the express themselves histologically and biologically. With regard t the nature of those defensive forces that study can tell us nothin positive, however; but it can at least give us the proofs of thei existence and of their actual working.

The fact that we find the same manner of reaction in naturall resistent mice and in those that have been artificially immunise by one or another of the processes described above, would seem t indicate that the question is, indeed, one of merely stimulatin the natural defensive forces of the organism.

In America, especially, considerable experimental work has bee done with a view to clearing up this problem, with the result tha the creation of immunity appears to be largely bound up wit the condition of the lymphoid tissue. Whether it is a question o direct action on the latter is very uncertain, however. All that ca be said is that after the implantation of cancer tissue there is ob served, both in the naturally resistant animals and in the immu nised ones:

1) hyperplasia of the lymphoid tissue;

2) an increase in the number of lymphocytes in the circulatin blood;

3) an accumulation of lymphocytes all around the neoplasti tissue.

Now, it has been found that an animal susceptible to cance will become more resistant to the implanted disease if exposed t influences capable of producing those lymphoid changes, to a mor or less marked extent, in the healthy organism; and that the sam influences, if brought to bear on a tumor-bearing animal will cause reduction, and sometimes the total disappearance of the tumor; an that, up to a certain limit, the degree of resistance will follow th degree of the influence brought to bear. If that limit is overpassed the organism will namely sometimes respond in exactly the contrar manner, and the resistance to the cancer be lowered instead.

The methods of immunisation used, besides those already mentioned, are:

1) injection of blood from an animal of the same species;
2) injection of blood from an animal of different species;
3) injection of olive oil and various fatty acids;
4) dry heat;
5) roentgen.

Da Fano (53) was one of the first to call attention to the important part played by the lymphocytes in connexion with the question of immunity to tumors. He described the observations he had made to that effect on animals immunised by means of tumor tissue, blood and embryonic skin.

Murphy and Morton (150) implanted cancer on animals previously immunised by the injection of defibrinated blood, and showed that there was a considerable increase in the number of lymphocytes twenty-four hours after such implantation had taken place. They found that the same effect occurred in the naturally resistent mice which they had used as controls; while in the cancer-susceptible control animals there was a slight drop in the number of lymphocytes. The latter observation is confirmed by the experiences of Mottram and Russ (140), and of Murphy, Nakahara (163, 165) and Sturm (152). The two last-named investigators have shown, besides, that the injection of defibrinated blood may stir up an increased activity in the nuclear centers of the lymphoid tissue, and that this activity may be still further increased by the implantation of cancer. In the case of the spleen, they assert that there may in this manner be produced a hyperplasia so constant that three weeks after the implantation there will be no difficulty in telling whether a specimen of that organ is from a mouse susceptible to cancer or from an immune one. Murphy, Hussey, Sturm and Nakahara (147) have injected the defibrinated blood of rats into mice, in order to study the effect produced by a heterologous protein. It seems that this alone does not produce immunity, but that a repeated injection at the same time as cancer tissue is implanted produces a good effect. The immunity thus obtained can be made to cease again by the application of a strong dose of roentgen either before or after the blood injection and implantation of cancer tissue.

Murphy and Sturm (152, 153, 154, 155) have investigated the effects of dry heat, and have shown that in mice it causes first a drop and shortly afterwards an increase in the number of lymphocytes, and that this hyperlymphocytosis makes the animals in

a large measure immune against implanted cancer, also where it is a case of a re-implanted spontaneous tumor.

NAKAHARA (159, 161, 162) has shown that olive oil and various fatty acids, when injected into the peritoneum in small doses, produce lymphocytosis and increase the resistance to cancer; while large doses — of olive oil, at least — have precisely the opposite effect.

TAYLOR, WITHERBEE and MURPHY (227), THOMAS, TAYLOR and WITHERBEE (229), NAKAHARA (158) and MURPHY (164, 166) have studied the effect of roentgen on the lymphoid tissue of various animals — monkeys, rabbits, cats, guinea pigs and mice — and have found that by giving small doses it is possible to increase the number of lymphocytes in the circulating blood. MURPHY and MORTON (150), MURPHY and NAKAHARA (166, 167, 168), MURPHY, NAKAHARA and STURM (151) have shown that this lymphocytosis in mice is accompanied by an increased resistance to implanted cancer tissue, but that the resistance ceases as soon as the lymphocytosis is suppressed by the application of large doses of roentgen. This is confirmed by the experiences of RUSS, CHAMBERS, SCOTT, MOTTRAM (38, 200,), KOK (104) and VALKEN (236). CHAMBERS, SCOTT and RUSS (38) have also shown that this favorable effect is the same on animals with fully developed tumors, even if the latter were kept covered during the irradiation; and KOK (104) found that also subsequently implanted tumor tissue refused to take, even in parts of the animal's body that had been thus protected with lead. MURPHY and MORTON (149) found that the same roentgen dose which produced immunity when applied directly to the body, did not make any difference in the percentage of takes from tumor tissue when applied to the latter *in vitro*.

Some of the agents that either increase or reduce the organism's resistance to cancer tissue have the same effect with regard to its resistance to bacteria. Thus, according to MURPHY and STURM (157), large doses of roentgen will reduce the capacity for the formation of the usual antibodies in rabbits, while dry heat will stimulate their development.

The contention of MURPHY, NAKAHARA (165) and APOLANT (11), that immunity against tumors depends in a large measure on the spleen, is denied by other writers. WOGLOM (252), for instance, asserts that at all events it cannot be a question of that organ always being larger in immune mice; it is simply that hitherto the natural variations in its size have not been sufficiently counted with. BULLOCK and ROHDENBURG (31) maintain that extirpation of the spleen in no way affects an acquired immunity or the retro-

gression, once started, of a tumor. They also — in contrast to APOLANT (11) — affirm that an animal from which the spleen has been removed is rendered immune just as easily as any other, and that the absence of the spleen does not make any difference as regards the percentage of takes of implanted tumors.

Also the bearing of the lymphocytosis on the question of immunity is a matter of controversy. SITTENFIELD (218, 219), at any rate, thinks he has proved that rats naturally immune to FLEXNER-JOBLING's (63) carcinoma do not lose that immunity when subjected to roentgen doses that considerably reduce the number of lymphocytes in the blood. In that contention he is supported by PRIME (187).

It is not a matter of indifference in what manner the lymphocytosis is produced. As SITTENFIELD has shown, the degree of resistance is not altered in the slightest, if the increase in the number of lymphocytes is brought about by the injection of pilocarpine or by the introduction into the veins of lymphocytes from other animals, in whom lymphocytosis has been produced beforehand by means of roentgen. From this it may be concluded that the presence of an increased number of lymphocytes is not in itself the direct reason of the immunity, but that the latter is determined by other conditions, of which the lymphocytosis is merely the indicator.

EARLIER INVESTIGATIONS CONCERNING THE GROWTH OF MALIGNANT TUMORS IN ROENTGEN-RAYED TISSUES

It may probably be taken for proved that it is possible by general roentgen irradiation to increase the resistance of the organism to a variety of tumors, at least when the latter are implanted subcutaneously. Another question is, whether it is possible by irradiation of a limited area to produce a purely local immunity, and whether the general effect resulting from an irradiation of that kind is so considerable that it will to any extent increase the degree of general resistance.

The first attempt to clear up this problem experimentally was made by FRANKL and KIMBALL (65), to whom the idea was suggested by the results of WASSERMANN's (239) investigations concerning the influence of radium on tumor tissue *in vitro*. FRANKL and KIMBALL used in their experiments only 12 animals. These were roentgen-rayed locally; 50 x being given, through 3 mm. of aluminum, distance: 18 cm., before the implantation was made. All the control animals developed large tumors; of the irradiated animals 4 did not develop any tumor at all, while the other 8 got only small, partly necrosing ones.

MURPHY (145), HUSSEY, NAKAHARA and STURM (146) experimented to the same effect, using pieces of Bashford's carcinoma no. 63, which they implanted either in or beneath the skin, after the latter had been given 1 HED of roentgen (spark, 3 inches; 10 milliampères; skin-to-focus distance, 6 inches; exposure period, 2 1/2 minutes; no filter). The implantation was done symmetrically with two pieces of tumor tissue, at corresponding points on each side of the animal's body; whereupon one side was kept covered during the irradiation, and thus served as control for the other. When the implantation had been done subcutaneously, no effect was observed whatsoever.

With intracutaneous implantation, when the latter was done seven days after the irradiation, the proportion of takes would be from 33.5 to 44.5 per cent. on the irradiated side, and from 90 to

100 per cent. on the protected one. The results varied but slightly from this when the implantation was done either 20 hours before the irradiation or 2 hours after it.

Irradiation of the tumor tissue *in vitro*, with the same dose of 1 HED, after it had been cut into small pieces, made no difference in the proportion of takes. The non-irradiated tissue grew in 18 mice out of 24; the irradiated in 16 of 24.

Histological examination showed a considerable lymphocytic infiltration of the irradiated areas, most markedly so after seven days.

These experiments of MURPHY, HUSSEY, NAKAHARA and STURM's were made over again by MURPHY, MAISIN and STURM (148) with a view to finding out whether the result of implantation in irradiated tissue after irradiation *in vitro* would be the same if a spontaneous carcinoma was used instead of Bashford's no. 63. For this purpose they used a spontaneous carcinoma of the breast, which they reimplanted on its original host after applying the desired dose of roentgen — in some of the experiments to the host, in others to the tissue *in vitro*. On the whole, the results were similar to those obtained by MURPHY, HUSSEY, NAKAHARA and STURM. By intracutaneous implantation, for instance, they got 38.6 per cent. of takes, as against 83.6 per cent. in the non-irradiated animals; besides, the rate of growth was considerably slower in the irradiated animals than in the non-irradiated ones. The tumor tissue irradiated *in vitro* grew just as rapidly, and in many cases more rapidly, than the non-irradiated; and the percentage of takes was not lowered. When the implanted tissue was roentgen-rayed *in situ*, the tumors disappeared in 76 per cent. of the cases, and the rest grew more slowly than those that had been irradiated *in vitro*. When a tumor was irradiated *in situ*, and a piece of it immediately afterwards implanted in a non-irradiated mouse, the percentage of takes remained unaltered.

MURPHY, MAISIN and STURM concluded that the dose of 1 HED unfiltered does not have any directly deteriorating effect on the cancer cells. That the tumor tissue is affected when the implantation is made directly *in* the roentgen-rayed skin, but not when it is implanted beneath it, they would explain by the fact that the accumulation of lymphocytes caused by roentgen doses of the strength they used is confined to the cutis and does not extend to the subcutaneous layers.

LIU, STURM and MURPHY (123) have tried to ascertain what influence the direct irradiation of subcutaneous tissue has on a tumor subsequently implanted in the latter. They loosened a square

flap of skin, and roentgen-rayed the underlying tissue directly. The dose is indicated by the following factors: spark, 3 inches; current, 20 milliampères; distance, 6 inches; exposure, 2 ½ minutes; which, according to their statement, should be equal to 1 HED. There were 33.9 per cent. of takes in the roentgen-rayed animals, as against 82.2 per cent. in the controls. It was particularly noticed that the tumors in the former showed a tendency to grow inwards, toward the abdominal cavity, while, at the same time, they projected so slightly above the skin that in some cases they couldn't be seen from the outside at all. When the tissue was implanted subcutaneously after the same dose had been applied only on the surface of the skin there was no difference between the roentgen-rayed and the non-roentgen-rayed animals, as regards the result. When a tumor was implanted in the right side of the animal after the subcutaneous tissue of the left side had been laid bare and irradiated directly, no effect was observed. Not until seven or nine days after the exposure had been made was any fairly considerable lymphocytic infiltration noticed in the irradiated tissue.

Kok (102) has studied — besides the results of implantation after general irradiation — the results of implantation in a field irradiated locally either immediately before or immediately afterwards, or else irradiated so long time after the implantation that the tumor already was fully developed. In the two first cases the best results were obtained with doses of from 225 to 275 *e.*; in the last with doses between 200 and 225 *e* (1 HED = 170 *e.*). In all three cases, the results were better when the irradiation was done over a field of from 2 to 2 ½ cm², than over a smaller field of only 7.5 × 15 mm. Kok and Vorlaender (106) ascribe the resorption of the tumor tissue very largely to the action of the histocytes.

In 1927, there were published two papers on this subject — one by Russ and Scott (202), the other by Beck and Engel (20). The two last-named, in order to throw light on the relation between the local tissue reaction and the part played by the general roentgen effect as regards the resorption of an implanted tumor, chose an experimental proceeding by which two similar tumors in the same animal were exposed to the same general action, but to different local ones. They accomplished this by implanting tumor fragments simultaneously in both hind legs after only a small field of one of them had been exposed to a dose of from 140 to 150 per cent. of 1 HED. The implantation was done, in the various animals, three, four and five days later. After three weeks, ten of the eleven mice that survived the operation showed tumors that were considerably larger in the non-irradiated leg than in the irradiated one.

In the eleventh, the tumors were of the same size in both legs; but in that animal the fragment implanted in the roentgen-rayed leg had fallen a little outside the irradiated area.

These results are similar to the ones obtained by MURPHY, HUSSEY, NAKAHARA and STURM (146) when the latter made their implantations in irradiated tissue intracutaneously. We have already said that their failure when the implantation was done subcutaneously was undoubtedly due, in part, to the quantity and quality of the radiation employed; but partly it may have been due, also, to a different malignancy of the tumors, and finally to the fact that they used the percentage of takes — while BECK and ENGEL used the size of the tumors at a given time — as measurement for the effect obtained.

RUSS and SCOTT (202) found that the tumors grew more slowly in such parts of the subcutaneous tissue as had been roentgen- or radium-rayed, than in those that had not. They covered all of the animal with lead, except two sector-shaped areas 9 millimeters distant from one another, and exposed for from 20 to 45 minutes. They used a Coolidge tube, and a spark length of 5 cm. between spheres 5 cm. in diameter. It took 20 minutes to produce epilation, 45 minutes to start vesiculation. The implantation was done either 3 days before the irradiation or, in other experiments, at various times after the exposure. They always found the growth to be strongest in the non-irradiated areas; the tumor gradually becoming flattened as it grew out into the roentgen-rayed tissue. Precisely the same experience was made when they irradiated for from 30 minutes to 1 hour, either before or after implantation, with 30 milligrams of radium bromide through 0.2 mm. of silver. Also here, the tumor avoided the irradiated area as long as possible, and became flatter than usual as soon as it began to invade the latter.

All these investigations have shown that it is possible to make a susceptible organism react to an implanted cancer in the same manner as a naturally resistant or an artificially immunised one. What one sees, is the formation of cells with phagocytic properties — lymphocytes and histocytes (macrophags) — and a resorption of the tumor tissue without any stroma being formed. There is some disagreement as to the exact time at which the immunising forces of the organism come into play. One possibility is that those forces are directed against the tendency of the tumor cells to chemotaxis, which causes the organism to react by the formation of a tumor stroma. If that is the case it might be imagined that the occurrence of the phagocytes is not the result of any specific reaction, but is simply the means by which the organism, in its natural way,

tries to get rid of an unwelcome foreign body — in this case the inserted tumor tissue. Another possibility is that, in the immune animals, there operates some factor which attracts the phagocytes and makes it possible for them to destroy the tumor cells.

It is RUSSELL (203, 204), particularly, who has made himself the advocate of the first of those theories. According to him, it cannot be a question of any directly destructive cell poison, inasmuch as, in resistant animals, the cancer cells can go on growing for as much as ten days, even in the peripheral portions of the tumor, where any poison developed in the harboring organism would be supposed to produce its greatest effect. At the same time, he dares not exclude the possibility of some toxic influence helping to weaken the cancer cells and, thus, assisting the natural defensive reaction of the organism.

RUSSELL's (203, 204) opinion is more or less shared by WOGLOM (250); but BASHFORD, MURRAY and CRAMER (17) are sceptical toward his theory, though unable to decide themselves whether the defensive reaction is directed against the specific stroma formation or has an immediate lethal effect on the tumor cells. D. FANO (53), like most of the American writers, holds that lymphocytes and plasma cells are largely instrumental in bringing about the resorption of the tumor, and points out that the organism of resistant animals is incapable of stroma formation; but he does not say anything as to what he believes to be the character of the defense, primarily. Isaac LEVIN (115) stands rather alone with his belief that immunity to tumors rests on the same conditions that determine immunity to bacteria.

In what manner the roentgen rays produce this immunising effect is not positively known; but it is not improbable that their action on the cells of the organism is responsible for the development of some activating function on the part of the latter. That the rays can cause certain changes in the chemical composition of the cellular protoplasm was shown already in 1903 and 1904 by SCHWARTZ (211, 212), SCHAPER (208) and WERNER (241). What those investigators specially laid weight on were the split-products of the lecithin; and WERNER (243, 244), ASCHER (245), and later, OPITZ (173, 176, 178), VORLAENDER and YOUNG (179), even used one of those split-products — namely, cholin and several of its compounds — as a remedy against cancer. It is curious to notice that, in Bohemia, cholin had already long been used as a popular cancer remedy; as a matter of fact, it was the knowledge of this that first gave OPITZ the idea to his experiments. That the irradiation causes the development of toxic products in the

rganism is sufficiently indicated by the story of the well-known »Röntenkater». Experimentally, it has been proved by ZACHERL (262), KOOSE (107), RAHM (188) and KOK (103), among others. ZACHERL howed that of two white rats that had been united parabiotically, t was possible to kill one by subjecting the other to a dose of roentgen; and he, like SCHWARTZ, SCHAPER, WERNER and OPITZ, believed hat the effect might possibly be due to the split-products of the ecithin. KOOSE (107, 188) has shown that, in all probability, the ffect of the rays is worked, not in the epithelium, but in the deeper ayers of the skin. He found that films of human skin, shaved off ccording to the method of THIERSCH and roentgen-rayed *in vitro*, vould stand a much stronger dose than the one which, applied to he skin *in situ*, produced ulceration of the latter. When the skin *n situ* was given the same dose, and was afterwards grafted onto nother animal in such a manner that the graft included the deeper ayers as well, that animal would die under symptoms of poisonng; but it could be saved if the graft was removed again in time. When the same piece was then, subsequently, grafted onto a third nimal, it was found to have lost its toxicity. KOOSE is rather nclined to believe that it is the intercellular medium, and not the ells, that are subject to the action of the rays. He mentions the act that BEHNE, when he transplanted roentgen-rayed skin to nother animal, would get the same lympho- and leucocytic reacions as the ones obtained by direct irradiation of the animal; while he transfusion of blood from a roentgen-rayed animal would proluce no such effect. KOK (103) has shown that the poisonous subtances formed can be directly neutralised by the cancer cells; and rom this he concludes that carcinoma mice can stand a larger lose of roentgen than healthy ones; and, reversely, that the life f a mouse that has been subjected to a lethal dose of roentgen nay be prolonged by the implantation of a carcinoma; and that, n such an animal, the carcinoma often will refuse to develop.

With regard to the nature of the active substances, CASPARI 32, 33, 34) has suggested that it might be a question of hormones — the products of necrosis or cellular degeneration — diffusing hemselves through the organism and stimulating its capacity for nhibition. Others, like BIER (24, 25) and THEILHABER (228), have hought the principal factor to be the local roentgen-inflammation, ossibly assisted by an increased activity of the blood-producing rgans. Others again have variously ascribed the inhibitory effect f the rays more particularly to their action on the reticulo-endohelial system (KOK, VORLAENDER (106)), on the endocrinous glands, he vegetative nerve system (OPITZ (174, 175, 176, 177), WICHELS

and Behrens (248), Rütz (207), Delle Piane and Badino (45), or to physical and chemical changes in the blood and lymph (Koos (107), Rahm (188), Zacherl (262), Wedd and Russ (240), Schmid (209), Fraenkel and Nitsniewitsch (64)).

Without discussing whether one or another of those theorie respecting the underlying cause is the right one, we may howeve take it for granted that Roussy (194) is correct in considering tha the occurrence of lymphocytes in considerable numbers in the imme diate neighbourhood of a tumor is the sign of a favorable reaction indicating that a defensive agent is present and exerting itsel

MY INVESTIGATIONS CONCERNING THE GROWTH OF MALIGNANT TUMORS IN ROENTGEN-RAYED TISSUE

At the time when I commenced the studies embodied in the present treatise, relatively few experiments had been made with a view to throwing light on the part which irradiation of the organism is capable of playing in the destruction of implanted malignant tumors. I therefore felt myself justified in taking up this question for examination; especially as to whether tumors possessing a various degree of malignancy reacted alike or differently in this respect.

The question as to the exact nature of the defensive powers wielded by the organism is so vast that I deemed it best to keep investigations into that problem entirely outside the scope of the present work. I have therefore made my experiments simply with a view to showing merely what happens, macroscopically, when tumor tissue is implanted either in the cutis or subcutaneously, after irradiation of the skin — without attempting to extend that examination either to the how or the why of that result as visible to the naked eye. The four questions which I intended my experiments to answer are:

1. — Does roentgen irradiation of the skin have any effect on a subsequently implanted, malignant tumor?

2. — Does it, in that respect, make any difference — as asserted by MURPHY, HUSSEY, NAKAHARA and STURM (146) — whether the implantation is done in the cutis or subcutaneously?

3. — Is the effect in case of local irradiation confined to the roentgen-rayed area, or does the local irradiation have a general effect capable of influencing a tumor tissue implanted in the non-irradiated skin, a considerable distance away?

4. — Does the length of the time that elapses between the day of irradiation and the day of implantation have any bearing on the results?

When I began the experiments, I chose a dose of approximately 1 HED strength, and the same filter that I had used for the irradiation experiments *in vitro*. As I found that the effect increased when I gradually made the doses stronger, I did not try with any doses below 1 HED, but rather made a series of careful experiments with doses up to as much as 6 S.-N., through 2 millimeters of aluminum. At that point, some of the animals died as the result of the irradiation; wherefore I made 6 S.-N. the top-limit of dosage. Most of the experiments were made with filtration through 2 mm of aluminum; only a very few were made without the use of any filter. The distance, in all cases, was 23 cm. As in the previous experiments, the duration of life, the proportion of takes and the size of the tumor after various lengths of time were used as indicators for the effect obtained. To these was added, as regards the sarcoma, a new consideration, to which I had not paid any attention before; namely, an infiltrative growth into the thoracic wall and a lesser tendency of the tumor to assume a spherical shape in the roentgen-rayed animals than in the controls. This is something similar to the tendency, observed by LIU, STURM and MURPHY of tumors implanted in roentgen-rayed subcutaneous tissue to grow into the abdominal cavity, but very little — or not at all — outward, toward the surface of the skin; and to the observation made by RUSS and SCOTT, that their growth is flatter in irradiated than in non-irradiated cutaneous connective tissue.

Also with regard to metastases, my examinations have only been concerned with such as were macroscopically visible; and as they have occurred very irregularly, and only in rare instances, I do not believe that any conclusions can be drawn, regarding them, from these experiments.

I begin with the carcinoma experiments. In the first series of these, the only dose used was of 5 S.-N., filtered through 2 mm of aluminum. It was the first dose I chose, when I began; and as the effect was immediately apparent — no takes in the irradiated animals, but takes in 50 per cent. of the controls — I concluded that I must have hit very nearly on the optimum of dosage.

In the first three experiments, a strip fully 1 cm. broad, across the anterior surface of the animal's thorax, just below the fore legs, was irradiated; in the two last ones, an area 1 × 1.5 cm. large

Fig. 2

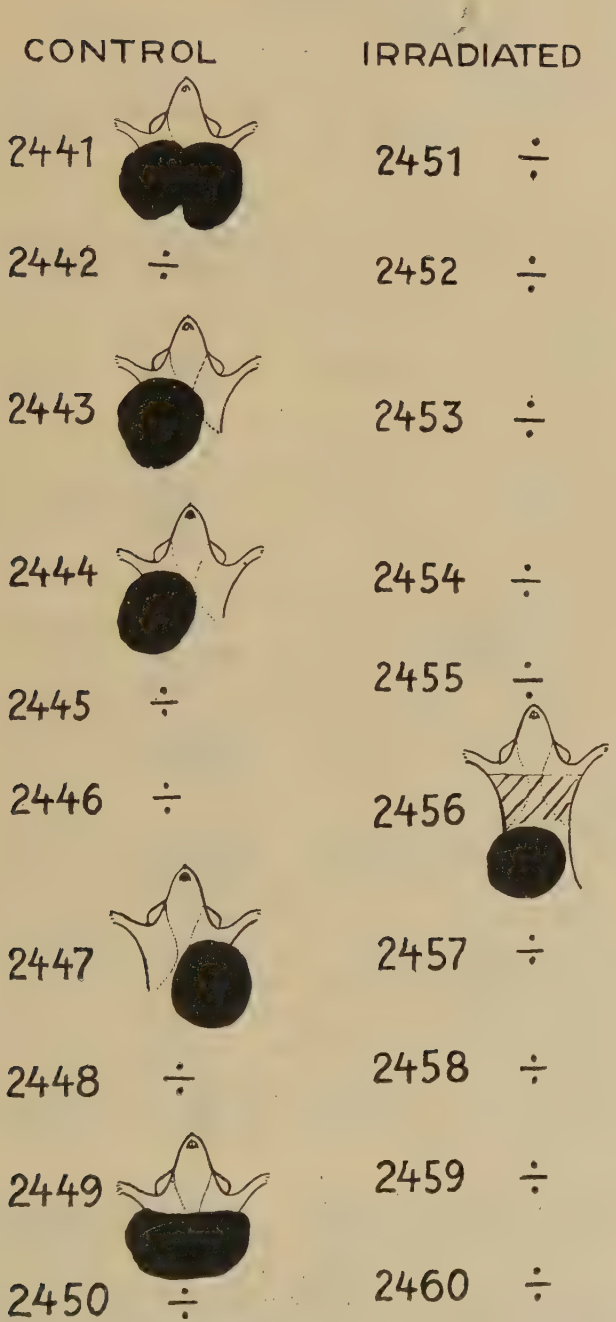


Fig. II. — The result, after about two months, of intracutaneous implantation of carcinoma in ten roentgen-rayed mice and ten controls. Dose: 5 S.-N.; filter: 2 mm. al.; distance: 23 cm.; field: 1 cm. broad, across anterior surface of thorax. Exposure and Implantation done on the same day, October 18th, 1926.

on the left side of the abdomen. In all cases, the rest of the bod was protected with a shield of 1 mm. of lead.

Experiment I comprises 20 animals, 10 of which were the con trols. The implantation was made intracutaneously, immediatel following the irradiation. In one of the irradiated animals, the frag ment must have accidentally been implanted outside the expose area; at least, there developed almost medially in the abdomen o this mouse a tumor exactly similar in size to those of the control I feel justified in leaving this »accident» out of the account. Th result then is, that the implantation did not take in a single on of the roentgen-rayed animals, while half of the controls develope large tumors (see Fig. II). As there was no take in any of the roen gen-rayed animals, calculation of the average duration of life omitted.

The dispositions for Experiment II were exactly the same, wit intracutaneous implantation immediately after the animals ha been roentgen-rayed. There were 4 takes among the ten roentgen rayed ones, and the average duration of life for the latter was 139 days. There were 8 takes among the ten controls, and the averag duration of life for these was 84 days.

Fig. III shows the markedly inhibitory effect on the tumo development in the roentgen-rayed animals. Table III shows th number of takes, and duration of life of each animal.

In Experiment III, the grafts were implanted — likewise intra cutaneously — seven days after the animals had been roentgen rayed. Positive result of the inoculation was obtained in fou mice out of ten which lived for an average of 99 days, while out o the ten controls six mice presented tumor growth, the average dura tion of their life being 85 days. One of the roentgen-rayed mice — no. 2827 — disappeared, though not until some time after a tumo had developed; in consequence, the average duration of life is cal culated only as regards the three others. The result of this experi ment shows that even with an interval of seven days between th roentgen exposure and the grafting, there is an undoubted inhib itory effect of the former on the subsequent tumor development The duration of life is so different in the individual animals in thi experiment (see Table IV) that it cannot serve as a true basis fo estimating the effect.

In Experiments IV and V (Fig. V & VI), the carcinoma graft were implanted intracutaneously at two symmetrically opposit points of the animals' abdomen, rather low down, after the lef side had been roentgen-rayed over a field 1 × 1.5 cm. in diameter In Experiment IV, the dose was of 5 S.-N., filtered through

Fig. 3

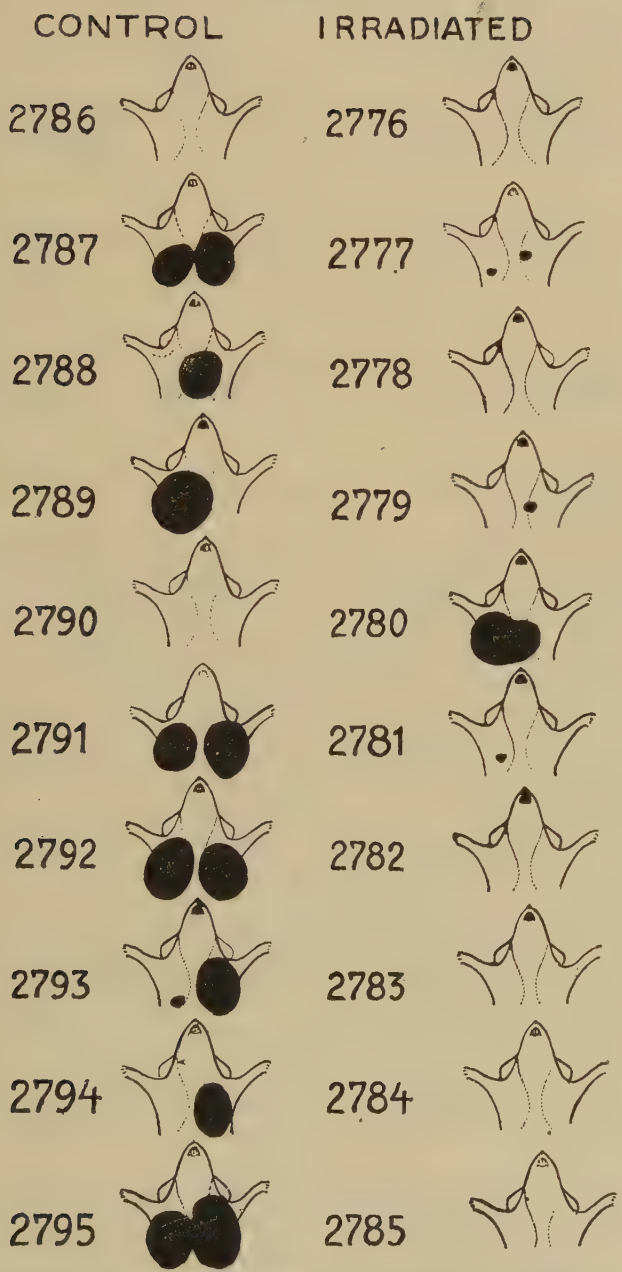


Fig. III. — The result, after about six weeks, of intracutaneous implantation of carcinoma in ten roentgen-rayed mice and ten controls. Dose: 5 S.-N.; filter: 2 mm. al.; distance: 23 cm.; field: 1 cm. broad, across anterior surface of thorax. Exposure and implantation on the same day, February 10th, 1927.

Experiment II *Table III*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2776	—	no take	2786	—	no take
2777	127		2787	77	
2778	—	no take	2788	73	
2779	164		2789	59	
2780	81		2790	—	no take
2781	187		2791	109	
2782	—	no take	2792	46	
2783	—	»	2793	110	
2784	—	»	2794	130	
2785	—	»	2795	68	
	559 : 4			672 : 8	
Average length of life: *139.8 days*			Average length of life: *84.0 days*		

mm. al.; distance, 23 cm.; in Experiment V, 3 S.-N., unfiltered same distance.

As, in these experiments, one half of the animal's body serve as control for the other half, the measurement of the effect by averag duration of life does not exist. The roentgen effect in these cases then, must be judged by the comparative periods of latency, rate of growth and proportions of takes. The experiments embraced in all, twenty animals. Three of these developed tumors in th roentgen-rayed side of the body; seven in the side that had bee protected from the rays. From the diagrams it will be seen tha the tumors developed in the exposed areas were decidedly retarde in their growth, as compared with those on the non-exposed side I had imagined that these experiments might serve to throw ligh on the question as to whether the local irradiation would hav any effect on a tumor subsequently implanted in a non-irradiate part of the same animal; but I afterwards realised that the contro had not been sufficient to allow of any valid conclusions bein made from them in that respect. In a more general way, howeve I think we may look at the result as indicating that the manne and rate of growth of a grafted tumor is exactly the same in th non-irradiated part of a locally roentgen-rayed animal as in a nor mal organism.

Finally, two experiments, nos. VI and VII, were made with im plantation of the carcinoma into the subcutaneous tissue; the roent gen dose in both being 5 S.-N., filtered through 2 mm. al.; dis tance, 23 cm.; field, 1 × 1.5 cm. The grafting was done seven day after the exposure had taken place.

Fig. 4

Fig. IV. — The result, after fully one month, of intracutaneous implantation of carcinoma in ten roentgenrayed mice and ten controls. Dose: 5 S.-N.; filter: 2 mm. al.; distance: 23 cm.; field: 1 cm. broad, across anterior surface of thorax. Exposure, February 26th, 1927; implantation, March 5th, 1927.

Experiment III *Table IV*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2819	—	no take	2809	—	no take
2820	—	»	2810	104	
2821	61		2811	74	
2822	—	no take	2812	89	
2823	122		2813	33	
2824	—	no take	2814	—	no take
2825	—	»	2815	—	»
2826	114		2816	120	
2827	—	out	2817	—	no take
2828	—	no take	2818	90	
	297 : 3			510 : 6	
Average length of life: *99.0 days*			Average length of life: *85.0 days*		

In Experiment VI, the number of takes was the same in the roentgen-rayed mice and in the controls; namely, fifteen out of twenty in each group. In Experiment VII, the grafting proved successful in eight out of seventeen roentgen-rayed animals, and in fifteen of the nineteen controls. The average duration of life was 74 and 117.6 days, respectively, for the roentgen-rayed ones; 48.5 and 89.1, respectively, for the controls (see Tables V and VI). Besides, as may be seen from the diagrams below, there is an undoubted effect on the period of latency and rate of growth in the roentgen-rayed animals. The results of these two experiments are found in Figs. VII and VIII, and in Tables V and VI.

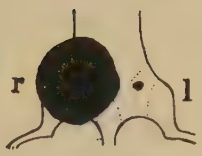
We thus observe not only a diminution of the growth energy, when the carcinoma is implanted intracutaneously, but also an indisputable lowering of the percentage of takes, such as was already noticed by MURPHY, HUSSEY, NAKAHARA and STURM. When the carcinoma is implanted subcutaneously, we find a similar inhibitory effect on the energy of growth; but, contrary to what is the case when the grafting is done intracutaneously, we do not here ascertain any definite lowering of the take-percentage; and this is in accordance with the observation made by BECK and ENGEL.

On the basis of the results of these experiments, the answers to the four questions formulated in the beginning of this chapter would be as follows:

1. — Roentgen irradiation of the skin has a positively demonstrable effect on a carcinoma subsequently implanted in the irradiated area. The manifestations of that ef-

Fig. 5

2548 r l



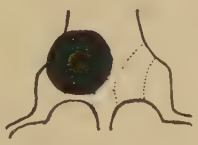
2549

2550 ÷

2551 ÷

2552 out

2553



2554 ÷

2555 ÷

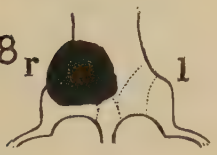
2556

2557 ÷

Fig. V. — Result, after about two months, of intracutaneous implantations of carcinoma simultaneously in a locally roentgen-rayed and a non-irradiated part of the same animal's body. Dose: 5 S.-N.; filter: 2 mm. al.; distance: 23 cm.; field: 1 × 1.5 cm. on left side of abdomen. Exposure, December 2nd, 1926; Implantation, December 9th, 1926.

Fig. 6

2568 r l



2569 ÷

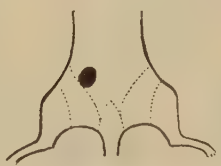
2570

2571 ÷

2572 ÷

2573

2574



2575 ÷

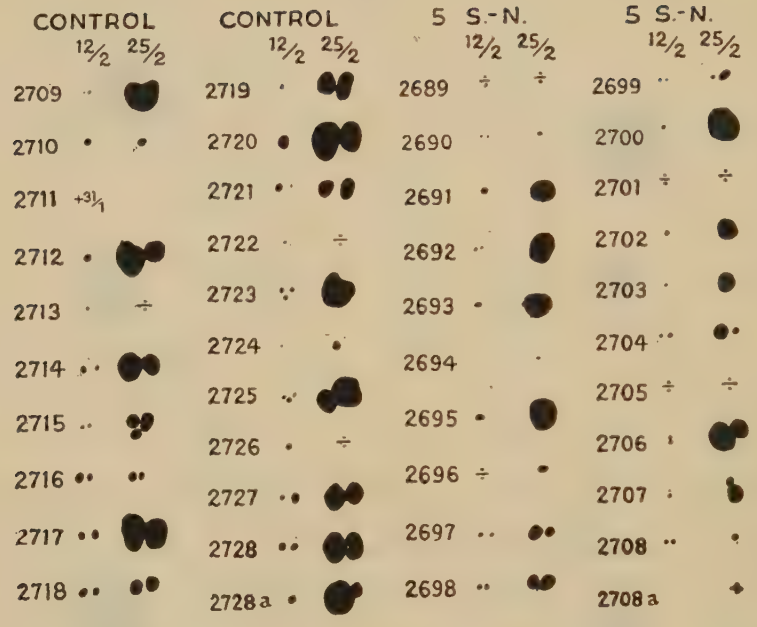
2576 ÷

2577 ÷

Fig. VI. — Result, after about two months, of intracutaneous implantations of carcinoma simultaneously in a locally roentgen-rayed and a non-irradiated part of the same animal's body. Dose: 3S.-N., no filter; distance, 23 cm.; field: 1 x 1.5 cm. on left side of abdomen. Exposure, December 2nd, 1926; implantation, December 9th, 1926.

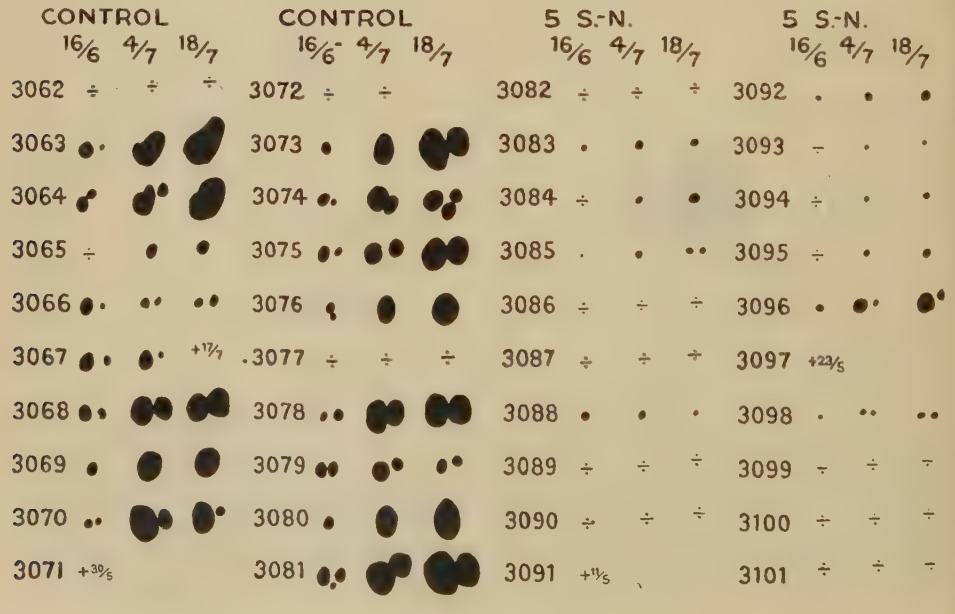
Experiment VI

Fig.7



Experiment VII

Fig.8



Experiment VI — *Table V*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2689	—	no take	2709	55	
2690	148		2710	—	no take
2691	58		2711	—	out
2692	51		2712	47	
2693	47		2713	52	
2694	—	no take	2714	42	
2695	—	no take	2715	51	
2696	94		2716	—	no take
2697	56		2717	39	
2698	59		2718	79	
2699	49		2719	45	
2700	60		2720	34	
2701	—	no take	2721	54	
2702	56		2722	—	no take
2703	103		2723	52	
2704	80		2724	—	no take
2705	—	no take	2725	46	
2706	59		2726	—	no take
2707	60		2727	42	
2708	130		2728	42	
2708 a	—	no take	2728 a	47	
	1110 : 15			727 : 15	
Average length of life: *74.0 days*			Average length of life: *48.5 days*		

Experiment VII — *Table VI*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
3082	—	no take	3062	—	no take
3083	165		3063	71	
3084	110		3064	81	
3085	133		3065	205	
3086	—	no take	3066	101	
3087	—	»	3067	57	
3088	—	»	3068	103	
3089	—	»	3069	86	
3090	—	»	3070	90	
3091	—	out	3071	—	out
3092	119		3072	—	no take
3093	—	no take	3073	65	
3094	—	out	3074	79	
3095	105		3075	78	
3096	58		3076	105	
3097	—	out	3077	—	no take
3098	107		3078	70	
3099	144		3079	—	no take
3100	—	no take	3080	76	
3101	—	»	3081	70	
	941 : 8			1337 : 15	
Average length of life: *117.6 days*			Average length of life: *89.1 days*		

fect are seen in the reduced energy of the tumor growth, in the reduced proportion of positive results, and in the average lengthening of the animal's life.

2. — The effect is most pronounced when the tumor has been implanted intracutaneously. It is less marked, but nevertheless indubitable, when implantation is made subcutaneously.

3. — Local irradiation has no macroscopically visible effect on a tumor subsequently implanted outside the roentgen-rayed area.

4. — As to whether variations in the intervals between the irradiation and the implantation might influence the effects of the roentgen rays in the case of intracutaneously implanted tumor, it is not possible to base any opinion upon the present experiments, because, in most of the cases, the applied roentgen dose proved so strong that there was no growth of the transplants whatever.

The next series of experiments were made with sarcomata. In these the experimental conditions were more varied, because it was found that, when it came to dealing with sarcomata, such doses as had been distinctly effective with regard to carcinoma did not produce any visible effect, at least as regards the proportion of takes and the average duration of the animal's life. In order to find out whether this was due to the doses or to the interval between irradiation and implantation, I have made my investigations bear on both those points.

In the Tables, the various degrees of the effect produced on the growth energy are indicated by the terms: *positive* (+), *doubtful* (?), *none* (0). With regard to the sarcomata, those expressions must, however, be taken with certain reservations. The diagrams show the circumference of the *visible* portion of the tumors, of course. It is therefore only in the cases where the latter are spherical, or nearly so, that this outline gives a more or less correct idea of their real size; while, in the cases where the tumor is more or less flattened or irregularly shaped, the circumference of the portion that can be felt through the skin does not furnish a truly dependable measure for the extent of the growth. It is thus by no means impossible

that a flattened tumor may at a certain stage of its development have an even larger circumference than a spherical one of the same age and endowed with the same energy of growth. The flat shape was observed in nearly all the tumors that grew in roentgen-rayed tissue, and most markedly so in those of the sarcomatous type; while the irregular shape was found particularly in such tumors as presented a pronounced tendency to downgrowth but never, so to speak, in the early stages of any of the tumors. In the diagrams, the circumference of the tumors is marked only as regards the early stages; and only in those cases where the flat tumors were smaller in circumference than the corresponding spherical tumors of the control animals have I taken this as an indication of a positive retarding effect. I believe that in this way I have provided against the possibility of any error of the kind alluded to.

In Experiments VIII, IX and X, the implantation was done intracutaneously; in the first one on the day of exposure, in the two others seven days after.

The results were as follows:

Table VII

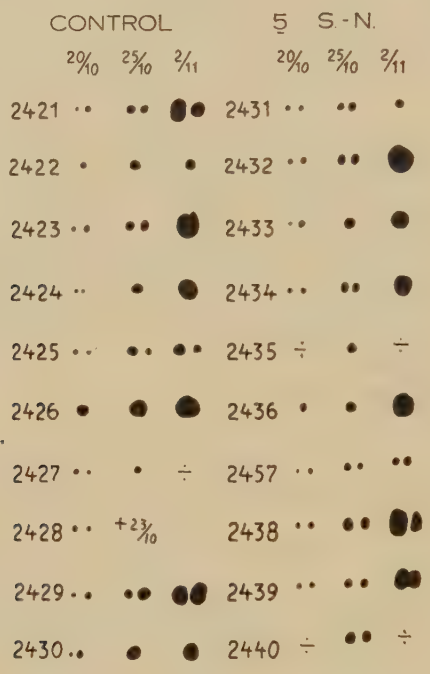
	Dose	Filter	Implantation	Average duration of life	Invasion of thorax	Effect on growth energy	Proportion of takes	Metastasis (macroscopically vis.)
Exp. VIII	5 S.-N.	2 mm. al.	same day	34.2 days	1	none	10/10	7
	Controls	—	—	37.9 »	0		9/10	8
Exp. IX.	2 S.-N.	none	7 days after	52.6 »	3	doubtful	15/19	12
	5 »	2 mm. al.	»	48.6 »	2	»	17/19	14
	Controls	—	—	47.4 »	0	—	17/20	11
Exp. X	2 S.-N.	none	7 days after	34.0 »	0	positive	11/12	6
	5 »	2 mm. al.	»	32.1 »	0	»	10/12	1
	Controls	—	—	28.3 »	1	—	12/12	3

The diagrams below give an idea of the size of the tumors and the rate of their growth, in each of the three experiments. The Tables

show the duration of life, invasion of thorax and occurrence of metastasis, for each animal. G = Invasion of thorax; M = metastasis.

Experiment VIII

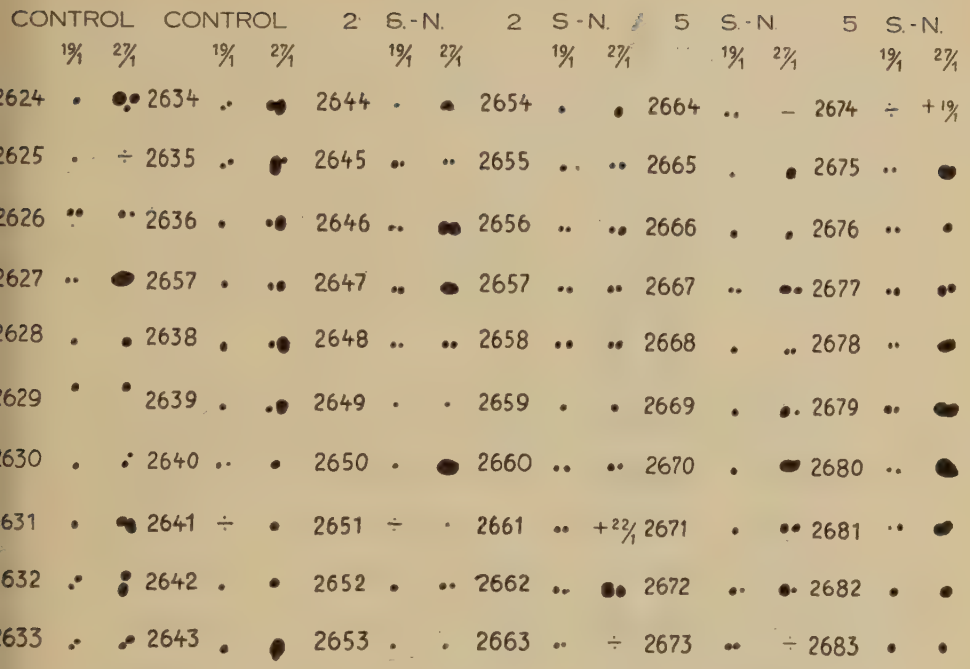
Fig. 9



Experiment VIII *Table VIII*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2431	34		2421	37	Metastasis
2432	34	Metastasis	2422	57	»
2433	26	»	2423	36	»
2434	30	»	2424	46	»
2435	42		2425	40	»
2436	34	Metastasis	2426	37	»
2437	42	»	2427	—	no take
2438	25	»	2428	9	
2439	37	»	2429	43	Metastasis
2440	38	Ingrowth	2430	36	»
	342 : 10			341 : 9	
Average length of life: *34.2 days*			Average length of life: *37.9 days*		

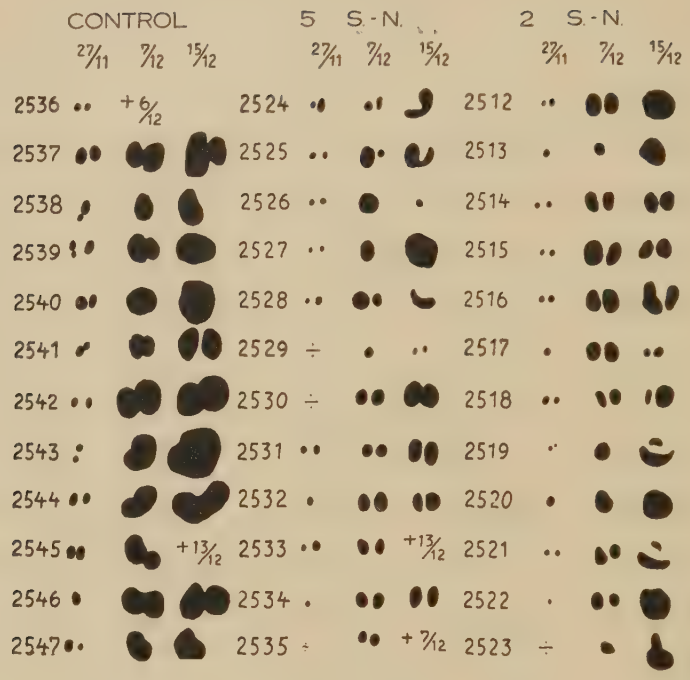
Experiment IX Fig. 10



Experiment IX *Table IX*

Roentgen-rayed mice						Controls		
2 S.-N., no filter			5 S.-N., 2 mm. aluminium					
Mouse No.	Life		Mouse No.	Life		Mouse No.	Life	
	days			days			days	
2644	52	Metastasis	2664	—	no take	2624	28	
2645	—	no take	2665	52		2625	—	no take
2646	45		2666	45	Metastasis	2626	45	Metastasis
2647	—	no take	2667	42	»	2627	54	
2648	—	»	2668	51	»	2628	68	Metastasis
2649	82	Metastasis	2669	68	Ingrowth. Metastasis	2629	—	no take
2650	45	Ingrowth. Metastasis	2670	46	Metastasis	2630	—	»
2651	65	Metastasis	2671	46	Ingrowth. Metastasis	2631	36	Metastasis
2652	61		2672	54	Metastasis	2632	26	
2653	50	Metastasis	2673	—	no take	2633	52	
2654	52	Ingrowth. Metastasis	2674	—	out	2634	54	Metastasis
2655	50	Metastasis	2675	56	Metastasis	2635	45	»
2656	41	»	2676	57		2636	39	»
2657	46	Ingrowth. Metastasis	2677	38	Metastasis	2637	47	»
2658	50		2678	35		2638	38	»
2659	53	Metastasis	2679	49	Metastasis	2639	39	»
2660	62	»	2680	41	»	2640	61	
2661	—	out	2681	44	»	2641	56	Metastasis
2662	35	Metastasis	2682	48	»	2642	58	»
2663	—	no take	2683	54	»	2643	59	
789 : 15			826 : 17			805 : 17		
Average length of life: *52.6 days*			Average length of life: *48.6 days*			Average length of life: *47.4 days*		

Experiment X Fig. 11



Experiment X *Table X*

Roentgen-rayed mice						Controls		
2 S.-N., no filter			5 S.-N., 2 mm. aluminum					
Mouse No.	Life		Mouse No.	Life		Mouse No.	Life	
	days			days			days	
2512	32	Metastasis	2524	41	Metastasis	2536	16	
2513	38	»	2525	32		2537	32	
2514	32		2526	—	no take	2538	36	
2515	34	Metastasis	2527	42		2539	30	Metastasis
2516	43	»	2528	44		2540	34	Ingrowth. Metastasis
2517	—	no take (small, rapidly disappearing tumor)	2529	—	no take	2541	32	
2518	36		2530	30		2542	28	
2519	35	Metastasis	2531	30		2543	27	
2520	32		2532	30		2544	28	
2521	35		2533	23		2545	23	Metastasis
2522	25		2534	32		2546	28	
2523	32	Metastasis	2535	17		2547	25	
374 : 11			321 : 10			339 : 12		
Average length of life: *34.0 days*			Average length of life: *32.1 days*			Average length of life: *28.3 days*		

It will be seen that when the implantation is done intracutaneously there is no effect on either the percentage of takes or the average duration of life, while in two instances there seems to be a positive retardation of the tumor's growth in the roentgen-rayed tissue. Not only does this appear from the lesser circumference of those tumors, but it will also be noticed that their shape is much flatter than that of the tumors in the control animals. In six of the roentgen-rayed animals the tumor had penetrated the thoracic wall. To the possible significance of this I shall come back later, in the following. Metastasis occurred in 58 per cent. of the controls, and in 65 per cent. of the roentgen-rayed animals.

If, now, we turn to the subcutaneously implanted sarcomata, we find that the results are only slightly different from those observed with the intracutaneously implanted ones, as will be seen from the following Table:

Table XI.

Exp. No.	Dose	Filter	Implantation	Average duration of life	Invasion of thorax	Effect on growth energy	Proportion of takes	Metastasis (macroscopically vis.)
XI	6 S.-N.	2 mm. al.	same day	32.1 days	11	positive	$\frac{16}{18}$	5
	Controls	—	—	29.5 days	1	—	$\frac{18}{18}$	8
XII	4 S.-N.	2 mm. al.	same day	30.3 days	14	doubtful	$\frac{20}{20}$	7
	Controls	—	—	31.9 days	0	—	$\frac{19}{19}$	3
XIII	2 S.-N.	2 mm. al.	2 days after	34.3 days	12	none	$\frac{26}{27}$	8
	Controls	—	—	36.1 days	0	—	$\frac{17}{18}$	3
XIV	4 S.-N.	2 mm. al.	5 days after	40.8 days	10	none	$\frac{18}{20}$	5
	Controls	—	—	39.6 days	1	—	$\frac{8}{10}$	1
XV	5 S.-N.	2 mm. al.	7 days after	33.9 days	10	positive	$\frac{20}{20}$	10
	Controls	—	—	24.0 days	0	—	$\frac{18}{18}$	3

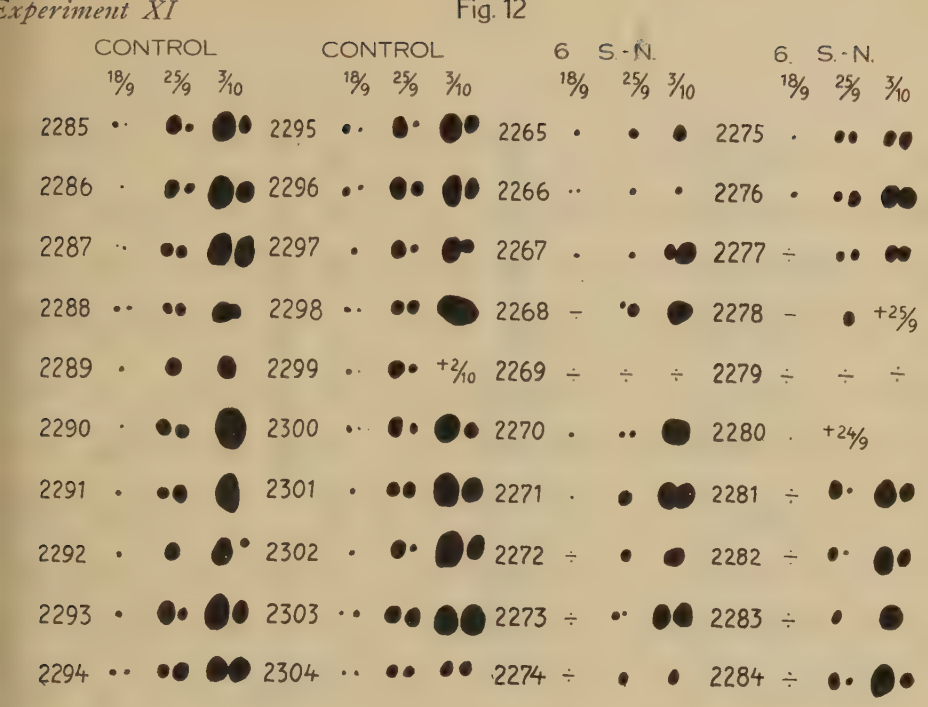
Table XI (cont.)

Exp. No.	Dose	Filter	Implantation	Average duration of life	Invasion of thorax	Effect on growth energy	Proportion of takes	Metastasis (marcoscopicall vis.)
XVI	5 S.-N.	5 mm. al.	7 days after	40.9 days	15	positive	20/20	10
	Controls	—	—	37.8 days	0	—	18/20	8
XVII	5 S.-N.	2 mm. al.	7 days after	30.4 days	9	positive	19/19	8
	Controls	—	—	26.9 days	3	—	20/20	7
XVIII	5 S.-N.	2 mm. al.	10 days after	41.2 days	5	positive	11/15	6
	Controls	—	—	35.8 days	0	—	11/12	0
XIX	5 S.-N.	2 mm. al.	10 days after	38.0 days	5	positive	12/12	4
	Controls	—	—	37.3 days	0	—	10/14	1

The figures below show the size of the tumors at different pe iods after implantation. The Tables give the duration of life fo each of the animals, and show the cases in which there was mac roscopical metastasis formation or invasive growth of the tumc into the thorax.

It will be noticed that in these ten last experiments, thoug in most cases the average duration of life of the roentgen-raye animals is a little longer — in one case (no. XV) even as much a 9.9 days longer — than that of the controls, the differences as re gards takes and average life-time, between the two groups, ar on the whole so slight that the results can hardly be taken as posi tive. If we look at this in connexion with the tendency of the tu mor in the roentgen-rayed animals to invade the thorax, it suggest itself that this tendency probably represents a new factor, capabl of compromising the life of the animal; and as it is but rarely me with in the controls, it is not unreasonable to infer that the lif of the roentgen-rayed animals might have been longer but for it presence. Among the subcutaneously inoculated animals it wa found in 56 per cent. of the roentgen-rayed ones, and in 3.6 pe cent. of the controls; among those inoculated intracutaneously

Experiment XI Fig. 12

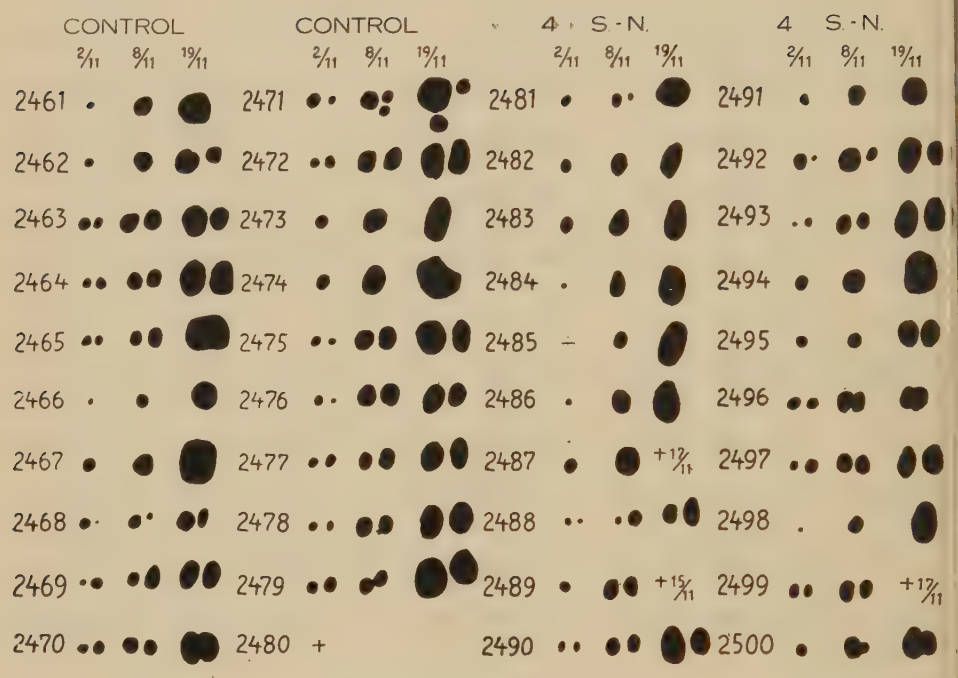


Experiment XI *Table XII*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2265	28	Ingrowth in thorax	2285	36	
2266	56	» » »	2286	32	Metastasis
2267	30	» » »	2287	32	»
2268	43	Ingrowth. Metastasis	2288	37	»
2269	—	out	2289	—	out
2270	23		2290	31	
2271	26	Ingrowth. Metastasis	2291	31	
2272	28	Ingrowth in thorax	2292	32	Metastasis
2273	25		2293	27	
2274	45		2294	29	
2275	25		2925	27	Metastasis
2276	32	Ingrowth. Metastasis	2296	32	
2277	32	Ingrowth in thorax	2297	27	Metastasis
2278	—	R+	2298	23	
2279	—	out	2299	21	
2280	—	R+	2300	26	Metastasis
2281	25	Ingrowth in thorax	2301	29	
2282	38	Ingrowth. Metastasis	2302	29	Ingrowth in thorax
2283	30	Metastasis	2303	30	
2284	27	Ingrowth in thorax	2304	—	out
	513 : 16			531 : 18	
Average length of life: *32.1 days*			Average length of life: *29.5 days*		

R + means that the death of the animal is due to the roentgen irradiation.

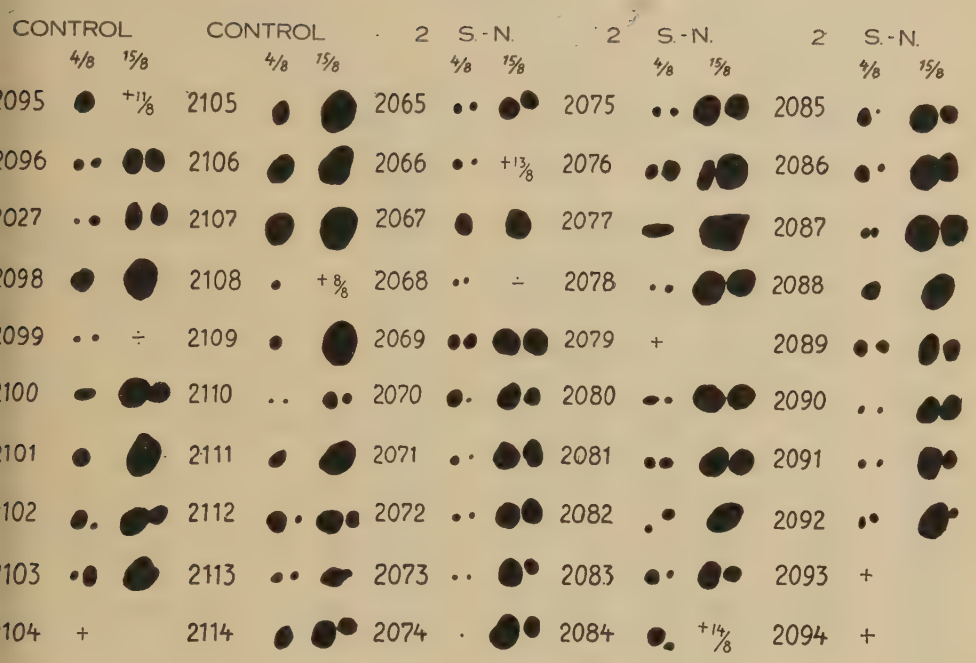
Experiment XII Fig. 13



Experiment XII *Table XIII*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2481	37	Ingrowth. Metastasis	2461	29	
2482	27	Ingrowth in thorax	2462	51	
2483	28	» » »	2463	31	
2484	29		2464	32	
2485	32	Ingrowth in thorax	2465	35	
2486	25	Ingrowth. Metastasis	2466	33	Metastasis
2487	29	Ingrowth in thorax	2467	33	
2488	33	Ingrowth. Metastasis	2468	37	
2489	23	Ingrowth in thorax	2469	30	Metastasis
2490	29		2470	34	
2491	28		2471	27	
2492	31	Metastasis	2472	30	Metastasis
2493	32		2473	26	
2494	37	Ingrowth in thorax	2474	33	
2495	33	» » »	2475	33	
2496	34	Ingrowth. Metastasis	2476	33	
2497	27	Ingrowth in thorax	2477	25	
2498	32	Ingrowth. Metastasis	2478	27	
2499	25	Metastasis	2479	28	
2500	34	Ingrowth	2480	—	out
	605 : 20			607 : 19	
	Average length of life: *30.3 days*			Average length of life: *31.9 days*	

periment XIII Fig. 14



n six roentgen-rayed animals and one control — the latter numbers being so small that I have not troubled to figure them out in terms of percentage. Metastasis was found in 24 per cent. of the controls, and in 39 per cent. of the roentgen-rayed animals; but as the average duration of life of the latter is at least *somewhat* longer, the actual difference becomes too slight to permit of any valid conclusions on that point. My impression, as regards the effect of irradiation on the subsequently implanted sarcoma, is that, in the cutis at least, it creates a certain resistance to the neoplastic growth, manifested, in the case of the intracutaneous graft, by a diminished energy of the tumor development, and, in the case of those subcutaneous grafts that grow into the cutis, by the fact that not only is the circumference of the tumor smaller, but also its shape is flatter than that of the tumors in the control animals. That the latter effect is limited to the irradiated area is evident from the fact that the tumors assume their usual rounded shape as soon as their growth begins to extend beyond that circumscribed field. The tumor used in these sarcoma experiments was of a much more virulent type than the one used in the carcinoma experiments, and it is there-

Experiment XIII *Table XIV*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2065	32		2095	23	
2066	25		2096	30	
2067	37		2097	71	
2068	—	no take	2098	41	Metastasis
2069	31	Ingrowth in thorax	2099	—	no take
2070	28		2100	32	Metastasis
2071	32	Ingrowth. Metastasis	2101	31	
2072	39	» »	2102	35	
2073	36	Ingrowth in thorax	2103	39	
2074	37	Metastasis	2104	—	out
2075	32	Ingrowth in thorax	2105	35	
2076	35		2106	30	
2077	30	Ingrowth. Metastasis	2107	40	
2078	35	Metastasis	2108	20	
2079	—	out	2109	34	
2080	32		2110	—	out
2081	39	Metastasis	2111	42	Metastasis
2082	39		2112	28	
2083	27		2113	40	
2084	26	Metastasis	2114	43	
2085	34	Ingrowth in thorax	.	.	
2086	33		.	.	
2087	35	Ingrowth in thorax	.	.	
2088	35	» » »	.	.	
2089	28	» » »	.	.	
2090	41	» » »	.	.	
2091	42	Ingrowth. Metastasis	.	.	
2092	51		.	.	
2093	—	out	.	.	
2094	—	»			
	891 : 26			614 : 17	
Average length of life: *34.3 days*			Average length of life: *36.1 days*		

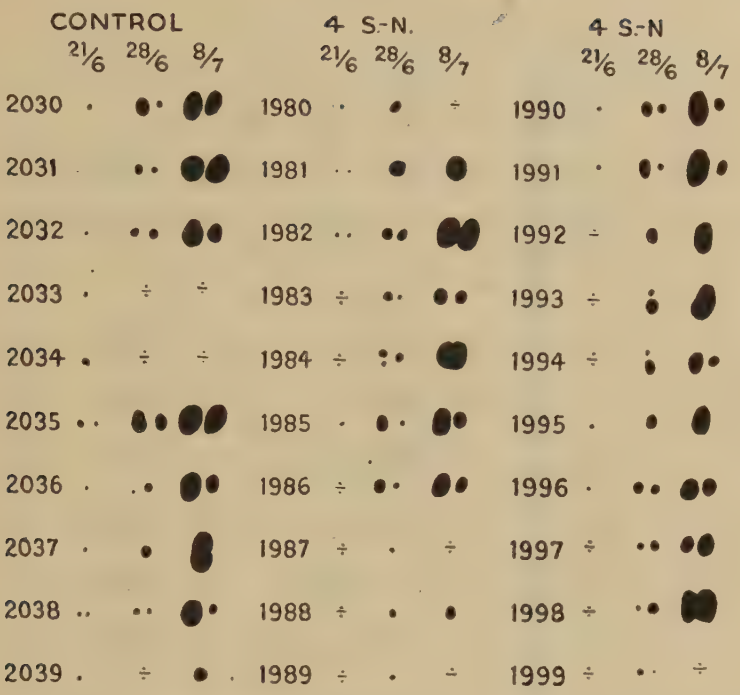
fore not to be wondered that the results are much less striking The answer to the questions formulated in the beginning of th present chapter would, as regards the sarcoma, be as follows:

1. — Roentgen irradiation of the skin de monstrably affects a sarcoma subsequentl implanted in the exposed area. The mani festation of that effect is seen in the reduce energy of the tumor growth. The proportio of takes is not reduced; nor is there an lengthening of the average duration of th animal's life.

2. — The effect appears to be confined t tumor tissue growing in the cutis itself.

Experiment XIV

Fig. 15



Experiment XIV — *Table XV*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
1980	73		2030	46	
1981	30	Ingrowth in thorax	2031	34	
1982	28	» » »	2032	35	
1983	39	» » »	2033	—	no take
1984	35	Ingrowth. Metastasis	2034	—	»
1985	28	Metastasis	2035	34	
1986	39		2036	43	Ingrowth in thorax
1987	—	no take	2037	41	Metastasis
1988	62		2038	42	
1989	—	no take	2039	42	
1990	33	Ingrowth in thorax	.	.	
1991	28	» » »	.	.	
1992	38	» » »	.	.	
1993	44	Ingrowth. Metastasis	.	.	
1994	39	Ingrowth in thorax	.	.	
1995	30	Metastasis	.	.	
1996	30		.	.	
1997	34	Ingrowth in thorax	.	.	
1998	37	Metastasis	.	.	
1999	88		.	.	
	735 : 18			317 : 8	
	Average length of life: *40.8 days*			Average length of life: *39.6 days*	

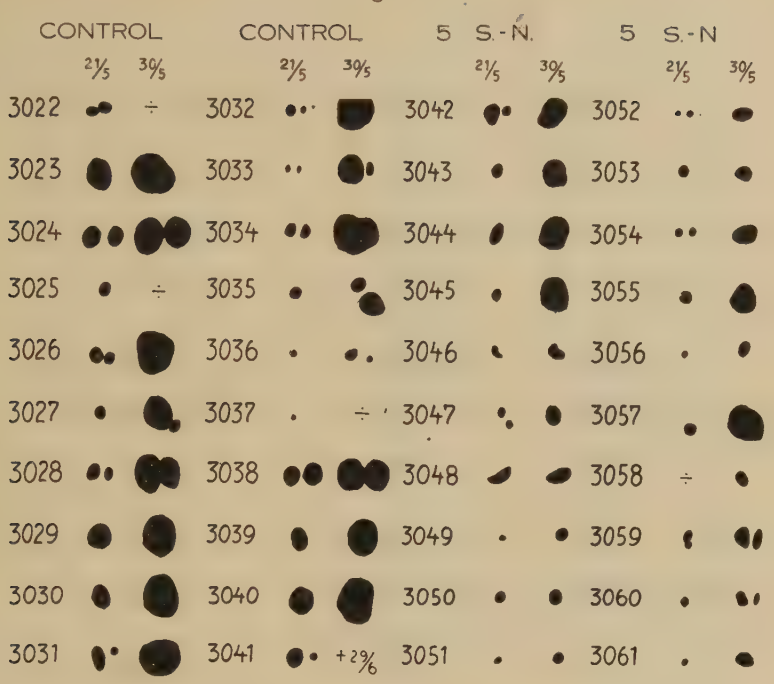
Experiment XV

Fig. 16

Experiment XV — *Table XVI*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2729	27	Ingrowth. Metastasis	2750	35	
2730	36		2751	31	Metastasis
2731	34	Ingrowth. Metastasis	2752	36	»
2732	35	Metastasis	2753	12	
2733	30	»	2754	12	
2734	22		2755	24	
2735	38		2756	36	
2736	—	out	2757	19	
2737	41	Ingrowth. Metastasis	2758	23	
2738	27	Metastasis	2759	23	
2739	30	Ingrowth. Metastasis	2760	16	
2740	28		2761	23	Metastasis
2741	41	Ingrowth in thorax	2762	23	
2742	39	» » »	2763	24	
2743	36	» » »	2764	23	
2744	30	Metastasis	2765	23	
2745	41		2766	27	
2746	29	Metastasis	2767	23	
2747	39	Ingrowth in thorax	2768	—	out
2748	36	Ingrowth. Metastasis	2769	—	»
2749	39	Ingrowth in thorax	2770	—	»
	678 : 20			433 : 18	
Average length of life: *33.9* days			Average length of life: *24.0 days*		

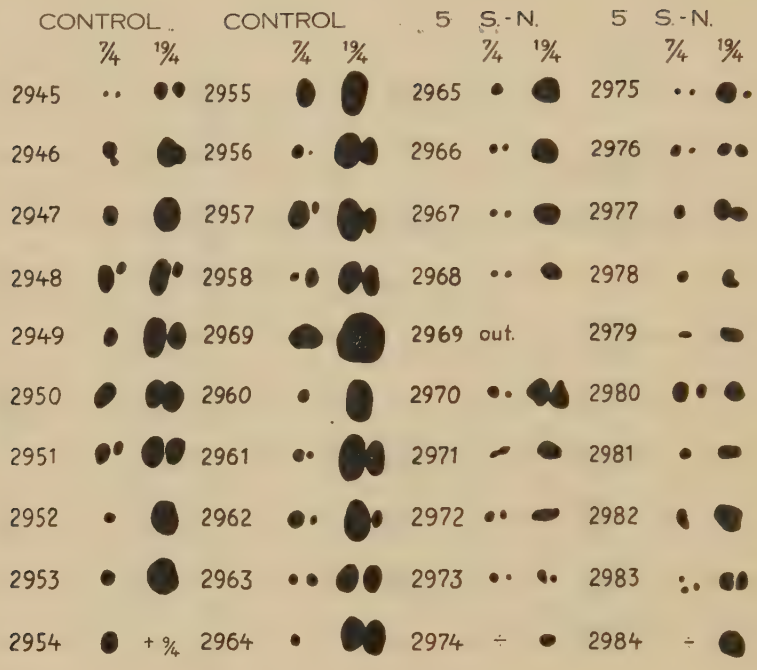
Experiment XVI Fig. 17



Experiment XVI *Table XVII*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
3042	41	Ingrowth in thorax	3022	—	no take
3043	54	Ingrowth. Metastasis	3023	36	
3044	34	Ingrowth in thorax	3024	48	
3045	34	Ingrowth. Metastasis	3025	—	no take
3046	41	» »	3026	31	
3047	33	Ingrowth in thorax	3027	44	
3048	35	» » »	3028	36	Metastasis
3049	61	Ingrowth. Metastasis	3029	34	
3050	41	» »	3030	40	Metastasis
3051	54	Ingrowth in thorax	3031	32	
3052	36	Metastasis	3032	40	
3053	39		3033	35	Metastasis
3054	51		3034	42	»
3055	32	Ingrowth. Metastasis.	3035	41	
3056	48	Metastasis	3036	41	
3057	23		3037	57	Metastasis
3058	43	Ingrowth in thorax	3038	35	»
3059	35	Ingrowth. Metastasis	3039	34	»
3060	40	» »	3040	33	»
3061	43	Ingrowth in thorax	3041	22	»
818 : 20			681 : 18		
Average length of life: *40.9 days*			Average length of life: *37.8 days*		

Experiment XVII Fig. 18



Experiment XVII *Table XVIII*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
2965	27	Metastasis	2945	48	Metastasis
2966	33		2946	23	
2967	47	Ingrowth in thorax	2947	28	Ingrowth in thorax
2968	25	» » »	2948	27	
2969	—	out	2949	24	
2970	25	Ingrowth. Metastasis	2950	24	Metastasis
2971	25	Metastasis	2951	27	»
2972	29		2952	25	Ingrowth. Metastasis
2973	53	Ingrowth in thorax	2953	24	
2974	47		2954	10	
2975	27	Metastasis	2955	29	
2976	34	Ingrowth in thorax	2956	28	Ingrowth in thorax
2977	28	Metastasis	2957	25	
2978	23	Ingrowth. Metastasis	2958	22	
2979	27	Metastasis	2959	20	Metastasis
2980	28	Ingrowth in thorax	2960	41	
2981	24	» » »	2961	26	Metastasis
2982	23	Ingrowth. Metastasis	2962	30	»
2983	25		2963	29	
2984	28		2964	28	
578 : 19			538 : 20		
Average length of life: *30.4 days*			Average length of life: *26.9 days*		

xperiment XVIII Fig. 19

CONTROL				5 S-N.			
	13/1	21/1	29/1		13/1	21/1	29/1
3285	••	●●	●●	3270	÷	•	●
3286	•	●	●	3271	••	●	●
3287	•	••	••	3272	•	÷	•
3288	•	•	•	3273	•	●	●
3289	••	●	●	3274	÷	•	÷
3290	•	••	●	3275	•	•	•
3291	•	•	•	3276	÷	•	•
3292	÷	••	●	3277	•	•	•
3293	•	÷	÷	3278	÷	÷	÷
3294	•	•	•	3279	÷	÷	÷
3295	•	••	••	3280	÷	•	•
3296	••	••	••	3281	•	•	•
3297	••	+16/1		3282	÷	÷	•
3298	••	•	•	3283	÷	•	÷
3299	•	+16/1		3284	•	÷	÷

Experiment XIX Fig. 20

CONTROL				5 S.-N.			
	14/1	22/1	22/1		14/1	22/1	22/1
3315	•	••	••	3300	÷	•	•
3316	••	•	•	3301	•	•	•
3317	••	••	••	3302	out		
3318	•	••	•	3303	out		
3319	•	••	••	3304	•	••	••
3320	•	••	••	3305	out		
3321	••	÷	÷	3306	•	••	••
3322	÷	••	••	3307	÷	••	•
3323	••	••	••	3308	•	•	•
3324	÷	•	÷	3309	•	•	••
3325	••	•	•	3310	•	÷	••
3326	••	•	÷	3311	÷	••	••
3327	•	••	••	3312	••	••	••
3328	•	•	•	3313	•	•	•
3329	••	••	•	3314	••	••	•

3. — As local irradiation had not had any demonstrable effect on a carcinoma implanted outside the irradiated area, I took it for granted that there would much less be any such effect on the far more virulent sarcoma under similar conditions, and therefore did not pursue that question farther as regards the latter.

4. — The effect seems to be strongest when the implantation is not done until some time after irradiation of the skin.

Experiment XVIII *Table XIX*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
3270	35	Metastasis	3285	40	
3271	43	Ingrowth. Metastasis	3286	—	out (take)
3272	40	» »	3287	38	
3273	24		3288	—	no take
3274	52		3289	38	
3275	37	Ingrowth. Metastasis	3290	22	
3276	57	Metastasis	3291	35	
3277	43	»	3292	43	
3278	—	no take	3293	—	no take
3279	—	no take	3294	38	
3280	52		3295	35	
3281	—	no take	3296	47	
3282	43	Ingrowth in thorax	3297	—	out
3283	—	no take	3298	22	
3284	47	Ingrowth in thorax	3299	—	out
	453 : 11			358 : 10	
	Average length of life: *41.2 days*			Average length of life: *35.8 days*	

Experiment XIX *Table XX*

Roentgen-rayed mice			Controls		
Mouse No.	Life		Mouse No.	Life	
	days			days	
3300	39	Ingrowth. Metastasis	3315	—	out
3301	42	Ingrowth in thorax	3316	38	
3302	—	out	3317	38	Metastasis
3303	—	»	3318	30	
3304	25		3319	38	
3305	—	out	3320	50	
3306	38		3321	—	no take
3307	48	Ingrowth. Metastasis	3322	48	
3308	48		3323	—	out
3309	34		3324	—	no take
3310	35	Metastasis	3325	30	
3311	42	»	3326	—	no take
3312	33	Ingrowth in thorax	3327	35	
3313	37	» » »	3328	43	
3314	35		3329	43	
	456 : 12			373 : 10	
	Average length of life: *38.0 days*			Average length of life: *37.3 days*	

From all that has been set forth in the foregoing it seems reasonable to suppose that the action of the roentgen rays is capable of releasing certain cancer-killing forces in the integument, extending sometimes to the subcutaneous tissue as well.

We find a substantiation of this, in the first place, in the results of the experiments made by MURPHY, NAKAHARA, HUSSEY and STURM, which showed that by the application of roentgen rays in rather feeble doses it is possible to retard, or even definitely prevent, the growth of a subsequently implanted carcinoma in the cutis — but not in the subcutaneous connective tissue.

We have, in the second place, the results of my own experiments, which show that under the same experimental conditions, but by using somewhat stronger doses, it is possible to obtain an inhibitory effect in the subcutaneous tissue as well — though the effect of any given dose will, of course, always be most pronounced in the cutis.

In the third place, we have seen that when my sarcoma was implanted in roentgen-rayed tissue, the inhibitory effect showed itself only as regards those fragments that were implanted intracutaneously; and, as regards the subcutaneously implanted fragments, only on those portions of the resulting tumor that grew into the cutis. The absence of any demonstrable effect on that portion of the tumor which grew into the subcutaneous tissue may be due to the fact that the virulence of this sarcoma was so great that all the inhibitory forces of the organism were spent in checking its development in the cutis — with exception, perhaps, of so small a part that their effect could not be measured by the standards I employed for the purpose. At any rate, I am unable to explain its growth away from the surface; nor have I found in any of the other writers any fully satisfactory explanation on this point. My idea on the subject is that it may possibly be interpreted as a defensive measure on the part of the sarcoma — an attempt to avoid the cancer-killing power at work in the skin and, perhaps, in the subcutaneous tissue. It is also possible, however, that the development of the tumor away from the surface may be due to a reduction of the local resistance in the deeper layers, as a result of the irradiation — or to the two causes combined.

Conditions of a similar nature are by no means unknown, clinically. Especially in cases of carcinoma of the breast it is sometimes observed, after a strong roentgen irradiation, that the development of the tumor will take an inward direction and that the tendency to metastasis will increase.

It is my hope that the views which I have expressed in the present work may perhaps to some extent explain the reason why so many diverging opinions have been advanced concerning the advisability or non-advisability of the roentgen treatment, especially as far as this particular type of cancer is concerned.

APPENDIX

TABLES SHOWING THE PROPORTION OF TAKES, AND THE AVERAGE AND INDIVIDUAL DURATION OF LIFE, IN THE VARIOUS EXPERIMENTS WITH IRRADIATION OF CARCINOMA AND SARCOMA IN VITRO AND IN SITU . . .

7—290464

Experiment I.

une 2nd, 1926.

Duration of life for 10 mice in whom had been implanted carcinoma from nouse no. 1843 after irradiation *in situ* with *3 S.-N.* through *2 mm. Al. at 23 m.* distance.

59 days	45 days
72 »	48 » + M
45 »	63 »
65 »	86 »
70 »	no take
311 : 5	242 : 4

Average duration of life: $\frac{553}{9} = 61.4$ days. Takes: 9 of 10.

Experiment II.

une 2nd, 1926.

Duration of life for 10 mice in whom had been implanted carcinoma from nouse no. 1835 after irradiation *in situ* with *6 S.-N.* through *2 mm. Al at 23 cm.* listance.

54 days	51 days
65 »	58 »
61 »	no take
no take	137 days
103 days + M	no take
283 : 4	246 : 3

Average duration of life: $\frac{529}{7} = 75.6$ days. Takes: 7 of 10.

Experiment III.

)ct. 10th, 1925.

Duration of life for 20 mice in whom had been implanted carcinoma from nouse no. 1835 after irradiation *in situ* with *6 S.-N.* through *2 mm. Al. at 23 cm.* listance.

91 days + M	80 days + M
83 » + M	no take
74 »	no take
118 »	no take
no take	no take
366 : 4	80 : 1
95 days	74 days + M
50 »	119 »
no take	no take
no take	no take
no take	no take
145 : 2	193 : 2

Average duration of life: $\frac{784}{9} = 87.1$ days. Takes: 9 of 20.

Experiment IV.

Dec. 15th, 1925.

Duration of life for 20 mice in whom had been implanted carcinoma fro mouse no. 1011 after irradiation *in situ* with *12 S.-N.* through *2 mm. Al.* in *cm.* distance.

no take	64 days + M
94 days	95 »
no take	no take
no take	no take
no take	no take
94 : 1	159 : 2
67 days	59 days
71 » + M	71 »
no take	134 »
no take	no take
no take	no take
138 : 2	264 : 3

Average duration of life: $\frac{655}{8} = 81.9$ days. Takes: 8 of 20.

Experiment V.

Oct. 4th, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma fro mouse no. 2135 after irradiation *in situ* with *12 S.-N.* through *2 mm. Al.* at *cm.* distance.

no take	215 days
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	eliminated

Average duration of life: $\frac{215}{1} = 215$ days. Takes: 1 of 19.

Experiment VI.

Jan. 9th, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma fro mouse no. 1396 after irradiation *in situ* with *15 S.-N.* through *2 mm. Al.* *23 cm.* distance.

146 days	no take
no take	no take
no take	no take
no take	no take
no take	no take
146 days	
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take

Average duration of life: $\frac{146}{1} = 146$ days. Takes: 1 of 20.

Experiment VII.

June 3rd, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma from mouse no. 1847 after irradiation *in situ* with *18 S.-N.* through *2 mm. Al at 23 cm.* distance.

no take	49 days
no take	106 »
no take	76 »
no take	no take
no take	no take
	231 : 3
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take

Average duration of life: $\frac{231}{3}$ = 77 days. Takes: 3 of 20.

Experiment VIII.

Oct. 4th, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma from mouse no. 2142 after irradiation *in situ* with *21 S.-N.* through *2 mm. Al. at 23 cm.* distance.

no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take

No takes in 20 animals.

Experiment IX.

Feb. 24th, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma from mouse no. 259 J. J. after irradiation *in vitro* with *3 S.-N.* through *2 mm. Al. at 23 cm.* distance.

117 days + M	46 days
71 »	no take
no take	no take
no take	no take
no take	no take
188 : 2	46 : 1
38 days	49 days
56 »	no take
51 »	no take
no take	no take
no take	no take
145 : 3	49 : 1

Average duration of life: $\frac{428}{7}$ = 61.1 days. Takes: 7 of 19.

Experiment X.

Feb. 24th, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma fro mouse no. 259 J. J. after irradiation *in vitro* with *6 S.-N.* through *2 mm. Al. 23 cm.* distance.

	94 days
eliminated	53 »
the cage	no take
broken	no take
	no take
	147 : 2
no take	93 days
81 days	74 »
no take	67 »
no take	no take
no take	no take
81 : 1	234 : 3

Average duration of life: $\frac{462}{6}$ = 77 days. Takes: 6 of 15.

Control for IX and X.

Feb. 24th, 1926.

100 days	no take
139 »	no take
no take	117 days + M
no take	no take
no take	no take
239 : 2	117 : 1
35 days	44 days
66 »	53 »
no take	101 » + M
170 days	81 »
no take	no take
271 : 3	279 : 4

Average duration of life: $\frac{906}{10}$ = 90.6 days. Takes: 10 of 20.

Average life of control animals in Exp. IX and X.

Experiment XI.

Dec. 10th, 1925.

Duration of life for 10 mice in whom had been implanted carcinoma fro mouse no. 1000 after irradiation *in vitro* with *9 S.-N.* through *2 mm. Al. 23 cm.* distance.

53 days	50 days
132 »	64 »
no take	76 » + M
no take	76 »
no take	no take
185 : 2	266 : 4

Average duration of life: $\frac{451}{6}$ = 75.2 days. Takes: 6 of 10.

Control.

+ tumor	+ tumor
+ tumor	+ tumor
+ tumor	+ tumor
+ tumor	no take
no take	no take

Average duration of life not stated, as the animals were used for therapeutical experiments. Takes: 7 of 10.

Experiment XII.

Dec. 12th, 1925.

Duration of life for 10 mice in whom had been implanted carcinoma from mouse no. 1237 after irradiation *in vitro* with *12 S.-N.* through *2 mm. Al. at 23 cm.* distance.

68 days	106 days
82 »	95 » + M
146 » + M	100 »
no take	120 »
no take	no take
296 : 3	421 : 4

Average duration of life: $\frac{717}{7} = 102.4$ days. Takes: 7 of 10.

Control.

+ tumor	eliminated
+ tumor	+ tumor
no take	+ tumor
no take	no take
no take	no take

Average duration of life not stated, as the animals were used for therapeutical experiments. Takes: 4 of 9.

Experiment XIII.

May 1st, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma from mouse no. 1637 after irradiation *in vitro* with *12 S.-N.* through *2 mm. Al. at 23 cm.* distance.

90 days	54 days
71 »	35 »
91 »	59 »
no take	no take
no take	271 days + M
252 : 3	419 : 4
68 days	eliminated
45 »	88 days + M
no take	no take
no take	no take
no take	no take
113 : 2	88 : 1

Average duration of life: $\frac{872}{10} = 87.2$ days. Takes: 10 of 20.

This experiment was made in connexion with Exp. XV and XVI, using the same control.

Experiment XIV.

Jan. 22nd, 1926.

Duration of life for 10 mice in whom had been implanted carcinoma fro: mouse no. 180 J. J. after irradiation *in vitro* with *15 S.-N.* through *2 mm. Al.* a *23 cm.* distance.

no take	no take	93 days
no take	no take	no take
no take	no take	no take
no take	no take	no take
no take	no take	no take
		93 : 1

Average duration of life: $\frac{93}{1}$ = 93 days. Takes: 1 of 15.

Control.

41 days	+ tumor (killed and tumor transplante
46 »	45 days
43 »	69 »
75 »	no take
no take	no take
205 : 4	114 : 2

Average duration of life: $\frac{319}{6}$ = 53.2 days. Takes: 7 of 10.

Experiment XV.

May 1st, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma fro: mouse no. 1637 after irradiation *in vitro* with *15 S.-N.* through *2 mm. Al.* a *23 cm.* distance.

69 days	91 days
87 »	no take
no take	no take
no take	no take
no take	no take
156 : 2	91 days : 1

92 days	140 days
no take	no take
no take	no take
no take	no take
no take	no take
92 : 1	140 : 1

Average duration of life: $\frac{479}{5}$ = 95.8 days. Takes: 5 of 20.

This experiment was made in connexion with Exp. XIII and XVI, using the same control.

Experiment XVI.

May 1st, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma from mouse no. 1637 after irradiation *in vitro* with *18 S.-N.* through *2 mm. Al. at 23 cm.* distance.

no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
134 days	103 days
192 »	no take
no take	no take
no take	no take
no take	no take
326 : 2	103 : 1

Average duration of life: $\frac{429}{3}$ = 143.0 days. Takes: 3 of 20.

This experiment was made in connexion with Exp. XIII and XV, using the same control.

Control for XIII, XV and XVI.

May 1st, 1926.

+ tumor	46 days
36 days	29 »
49 »	+ tumor
38 »	+ tumor
no take	no take
123 : 3	75 : 2
35 days	29 days
no take	38 »
+ tumor	43 »
35 days	32 »
no take	+ tumor
70 : 2	142 : 4

Average duration of life: $\frac{410}{11}$ = 37.3 days. Takes: 16 of 20.

Average life of control animals in Exp. XIII, XV and XVI.

Experiment XVII.

July 31st, 1926.

Duration of life for 20 mice in whom had been implanted carcinoma from mouse no. 2061 after irradiation *in vitro* with *21 S.-N.* through *2 mm. Al. at 23 cm.* distance.

no take	no take	no take	no take
no take	no take	no take	no take
no take	no take	no take	no take
no take	no take	no take	no take
no take	eliminated	no take	no take

No takes in 19 animals.

Control.

+ tumor	+ tumor + M
+ tumor	+ tumor
65 days	+ tumor
no take	no take
no take	no take
65 : 1	

Average duration of life: $\frac{65}{1} = 65$ days. Takes: 6 of 10.

Experiment XVIII.

June 17th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1936 after irradiation *in situ* with *3 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

30 days + M	39 days
39 »	41 »
32 »	45 » + M
36 » + M	47 »
80 »	39 » + M
217 : 5	211 : 5

Average duration of life: $\frac{428}{10} = 42.8$ days. Takes: 10 of 10.

Experiment XIX.

June 17th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1937 after irradiation *in situ* with *6 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

30 days	38 days
26 »	34 »
40 »	75 » + M
37 » + M	47 » + M
29 »	71 »
162 : 5	265 : 5

Average duration of life: $\frac{427}{10} = 42.7$ days. Takes: 10 of 10.

Experiment XX.

Nov. 24th, 1925.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1177 after irradiation *in situ* with *9 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

50 days	58 days
45 »	116 »
48 »	no take
no take	no take
no take	no take
143 : 3	174 : 2

Average duration of life: $\frac{317}{5} = 63.4$ days. Takes: 5 of 10.

Experiment XXI.

Jan. 27th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1375 after irradiation *in situ* with *12 S.-N.* through *2 mm. Al. at 23 cm.* distance.

no take	86 days
no take	74 »
no take	73 »
no take	87 »
no take	no take
	320 : 4

Average duration of life: $\frac{320}{4}$ = 80 days. Takes: 4 of 10.

Experiment XXII.

Jan. 22nd, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1471 after irradiation *in situ* with *15 S.-N.* through *2 mm. Al. at 23 cm.* distance.

no take	37 days
40 days	80 »
68 »	no take
72 »	no take
no take	no take
180 : 3	117 : 2

Average duration of life: $\frac{297}{5}$ = 59.4 days. Takes: 5 of 10.

Experiment XXIII.

Oct. 2nd, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 2306 after irradiation *in situ* with *18 S.-N.* through *2 mm. Al. at 23 cm.* distance.

79 days + M	no take
no take	no take
no take	144 days
no take	no take
no take	no take
79 : 1	144 : 1

no take	no take
no take	no take
no take	no take
no take	no take
no take	eliminated

Average duration of life: $\frac{223}{2}$ = 111.5 days. Takes: 2 of 19.

Experiment XXIV.

Sept. 2nd, 1926.

Duration of life for 20 mice in whom had been implanted sarcoma from mouse no. 2172 after irradiation *in situ* with *21 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
no take	no take

No takes in 20 animals.

Experiment XXV.

Feb. 2nd, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 318 J. J. after irradiation *in vitro* with *3 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

no take	35 days
no take	36 »
69 days	53 »
no take	35 »
no take	36 »
69 : 1	195 : 5

Average duration of life: $\frac{264}{6}$ = 44 days. Takes: 6 of 10.

Experiment XXVI.

Apr. 24th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1750 after irradiation *in vitro* with *3 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

29 days	32 days
25 »	25 »
27 »	27 »
25 »	27 »
25 »	14 »
131 : 5	125 : 5

Average duration of life: $\frac{256}{10}$ = 25.6 days. Takes: 10 of 10.

Experiment XXVII.

Feb. 10th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 318 J. J. after irradiation *in vitro* with *6 S.-N.* through *2 mm. at 23 cm.* distance.

36 days	48 days
41 »	47 »
126 »	40 »
41 »	38 »
64 »	no take
308 : 5	173 : 4

Average duration of life: $\frac{481}{9}$ = 53.4 days. Takes: 9 of 10.

Control XXV and XXVII.

Feb. 10th, 1926.

44 days	19 days
31 »	22 »
31 »	26 »
32 »	26 »
eliminated	26 »
138 : 4	119 : 5

Average duration of life: $\frac{257}{9}$ = 28.6 days. Takes: 9 of 9.

Experiment XXVIII.

Apr. 24th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1750 after irradiation *in vitro* with *6 S.-N.* through *2 mm. Al. at 23 cm.* distance.

36 days	26 days
41 »	30 »
38 »	18 »
16 »	26 »
no take	no take
131 : 4	100 : 4

Average duration of life: $\frac{231}{8}$ = 28.9 days. Takes: 8 of 10.

Experiment XXIX.

Dec. 7th, 1925.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1287 after irradiation *in vitro* with *9 S.-N.* through *2 mm. Al. at 23 cm.* distance.

38 days	+ tumor (died from other causes)
32 »	49 days
no take	81 »
67 days	no take
no take	49 days
137 : 3	179 : 3

Average duration of life: $\frac{316}{6}$ = 52.7 days. Takes: 7 of 10.

Control.

+ tumor	+ tumor
+ tumor	+ tumor
+ tumor	+ tumor
+ tumor	31 days
+ tumor	31 »
	62 : 2

Average duration of life: $\frac{62}{2}$ = 31 days. Takes: 10 of 10.

Animals afterwards used for therapeutical experiments.

Experiment XXX.

Apr. 24th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1750 after irradiation *in vitro* with *9 S.-N.* through *2 mm. Al. at 23 cm.* distance.

38 days	32 days
38 »	34 »
32 »	41 »
29 »	34 »
32 »	no take
169 : 5	141 : 4

Average duration of life: $\frac{310}{9}$ = 34.4 days. Takes: 9 of 10.

Experiment XXXI.

Sept. 5th, 1926.

Duration of life for *25* mice in whom had been implanted sarcoma from mouse no. 2168 after irradiation *in vitro* with *9 S.-N.* through *2 mm. Al. at 23 cm.* distance.

42 days	47 days	eliminated
51 » + M	46 »	43 days
60 »	39 »	46 »
64 »	67 »	68 »
no take	no take	no take
217 : 4	199 : 4	157 : 3

39 days + M	+ tumor
47 »	53 days
34 »	34 »
83 » + M	34 »
no take	84 »
203 : 4	205 : 4

Average duration of life: $\frac{981}{19}$ = 51.6 days. Takes: 20 of 24.

Experiment XXXII.

Jan. 13th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 112 J. J. after irradiation *in vitro* with *12 S.-N.* through *2 mm. Al. at 23 cm.* distance.

no take	no take
no take	no take
no take	no take
no take	no take
no take	no take
No take.	

Control.

+ tumor	+ tumor
26 days	+ tumor
+ tumor	+ tumor
23 days	+ tumor
+ tumor	+ tumor
no take	+ tumor
+ tumor	+ tumor
+ tumor	eliminated
+ tumor	+ tumor
eliminated	+ tumor

Tumor used for other experiments. Takes: 17 of 18.

Experiment XXXIII.

Apr. 24th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1750 after irradiation *in vitro* with *12 S.-N.* through *2 mm. Al. at 23 cm.* distance.

53 days	34 days
34 »	35 »
no take	39 »
no take	41 »
no take	39 »
87 : 2	188 : 5

Average duration of life: $\frac{275}{7} = 39.3$ days. Takes: 7 of 10.

Experiment XXXIV.

Apr. 24th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1750 after irradiation *in vitro* with *15 S.-N.* through *2 mm. Al. at 23 cm.* distance.

32 days	38 days
51 »	41 »
53 »	no take
51 » + M	no take
46 »	no take
233 : 5	79 : 2

Average duration of life: $\frac{312}{7} = 44.6$ days. Takes: 7 of 10.

Control for XXVI, XXVIII, XXX, XXXIII and XXXIV.

Apr. 24th, 1926.

+ tumor + M	32 days
27 days	27 »
25 »	22 »
21 »	31 »
30 »	eliminated (died immediatel after the operation)
103 : 4	112 : 4
27 days	30 days
+ tumor	27 » + M
31 days	26 »
+ tumor	20 »
eliminated (died 5 days after the operation)	25 »
58 : 2	128 : 5

Average duration of life: $\frac{401}{15} = 26.7$ days. Takes: 18 of 18.

XXVI: 3 S.-N. 2 mm. Al. $\frac{256}{10} = 25.6$ days. Takes $\frac{10}{10}$

XXVIII: 6 S.-N. 2 mm. Al. $\frac{231}{8} = 28.9$ days. Takes $\frac{8}{10}$

XXX: 9 S.-N. 2 mm. Al. $\frac{310}{9} = 34.4$ days. Takes $\frac{9}{10}$

XXXIII: 12 S.-N. 2 mm. Al. $\frac{275}{7} = 39.3$ days. Takes $\frac{7}{10}$

XXXIV: 15 S.-N. 2 mm. Al. $\frac{312}{7} = 44.6$ days. Takes $\frac{7}{10}$

Experiment XXXV.

Oct. 2nd, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 2305 after irradiation *in vitro* with *15 S.-N.* through *2 mm. Al.* at *23 cm.* distance.

no take	no take
no take	no take
no take	no take
no take	no take
no take	no take

No takes in 10 animals.

Experiment XXXVI.

May 15th, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 1826 after irradiation *in vitro* with *18 S.-N.* through *2 mm. Al. at 3 cm.* distance.

no take	51 days
no take	no take
no take	no take
no take	no take
no take	no take
	51 : 1

Average duration of life: $\frac{51}{1} = 51$ days. Takes: 1 of 10.

Experiment XXXVII.

Oct. 2nd, 1926.

Duration of life for 10 mice in whom had been implanted sarcoma from mouse no. 2305 after irradiation *in vitro* with *21 S.-N.* through *2 mm. Al. at 3 cm.* distance.

no take	no take
no take	no take
no take	no take
no take	no take
no take	eliminated

No takes in 10 animals.

Control for XXXV and XXXVII.

Oct. 2nd, 1926.

30 days	23 days
+ tumor	29 » + M
+ umor	24 » + M
3 days	21 »
+ tumor	24 »
61 : 2	121 : 5

23 days + M	28 days
25 » + M	16 »
30 » + M	16 »
31 » + M	24 » + M
no take	28 »
109 : 4	112 : 5

Average duration of life: $\frac{403}{16} = 25.2$ days. Takes: 19 of 20.

Metastasis formation and duration of life for a series of sarcoma-contr mice in the time from Jan. 1st 1926 to Jan. 1st 1927.

Duration of life	Duration of life	Duration of life	Duration of life
26 + M	30 + M	61	25
29 + M	34 + M	46	21
29	32	61	30
30	28	30	32
30	27	26	27
31	28	24	22
23	23 + M	18 + M	31
29 + M	28	13	27
24 + M	25	21	31
21	21	21	30
24	21	40 + M	27 + M
23 + M	20	51	26
25 + M	20	75 + M	20
30 + M	28	77 + M	25
31 + M	28	77	43
28	45 + M	42	45
16	54	18	41
16	68 + M	31	29
24 + M	36	31	30
28	26	40	29
37 + M	52	50	38
57 + M	54 + M	26	36 + M
36 + M	45 + M	23	21
46 + M	39 + M	44	25
40 + M	47 + M	31	25
37 + M	38 + M	31	30 + M
43 + M	39 + M	32	30 + M
36 + M	61	19	29
29	56 + M	22	32
51	58 + M	26	37
31	59 + M	26	33
32	23	19	28
35	35	33	38
33 + M	31	42	18
36	36 + M	47	32
37	12	33	40 + M
30 + M	12	48	26
37	24	30	51
27	36 + M	35	41
30 + M	19	48	22
26	23	25	36
33	23	27	32 + M
34	16	31	32 + M
33	23 + M	39	32 + M
25	23	28	31
27	24	28	31
28	23	36	32
30	23	37	27
25	27	39	29
36 + M	23	15	27 + M
26	24	31	32
16	30	38	27 + M
32	30	42	23
36	30	27	21

intracutaneously

Average duration of life for mice transplanted subcutaneously with sarcoma: $\frac{5500}{180} = 30.6$ days

Average » » » » » » intracutaneously » » $\frac{1476}{37} = 39.9$ days

Metastasis formation and duration of life for a series of carcinoma-control mice in the time from Jan. 1st 1926 to Jan. 1st 1927.

Duration of life	
102	
56	
120	
82	
58	
201	
122 + M	
228 + M	
107	
66	
150 + M	
61	
41	
46	
43	
75	
45	
69	
47	
59	
44	
51	
56	
45	
50	
61	
44	
100	
139 + M	
117	
35	
66	
170	
44	
53 + M	
101	
81	
36	
49	
38	
46	
29	
35	
35	
29	
38	
43	
32	
69	
96	
64	
47	
43	
43	
65	
40	
36	
53	
80	intracutaneously
140	intracutaneously
82	intracutaneously
122	intracutaneously
55	
40	
42	
51	
39	
45	
34	
54	
52	
46	
42	
42	
47	

Average duration of life for mice transplanted subcutaneously with carcinoma:

$\frac{4590}{71} = 64.6$ days.

RÉSUMÉ

Hensigten med det foreliggende Arbejde har været at undersøge følgen Spørgsmaal:

1. Hvor stor Røntgendosis skal der anvendes for at opnaa en direkte dr bende Virkning paa Kræftceller?

2. Kan man paavise at ikke dræbende Doser har nogen Virkning paa Kræ celler?

3. Er det muligt ved lokal Røntgenbestraaling at forøge eller nedsætte Værtsorganismes Modstandskraft over for Kræftvæv implanteret intra- ell subcutant i det bestraalede Areal?

Forsøgsdyrene har været Mus, og der er benyttet et Carcinom og et Sarcor af hvilke Sarcomet er det mest maligne, begge er opstaaet hos mine egne Dy Carcinomet er et spontant Mammacarcinom. Sarcomet er opstaaet efter Tjær pensling af Huden.

Røntgenstraalernes direkte Virkning paa Kræftcellerne er undersøgt dels ve at bestraale Svulstvæv in vitro og implantere det subcutant straks efter B straalingen, dels ved at bestraale Svulster in situ og transplantere Stykker a dem til andre Dyr, umiddelbart efter Bestraalingen.

Som Maal for Paavirkningens Grad er benyttet:

Anslagsprocenten.

Dyrenes Levetid efter Transplantationen.

Latenstiden og Vækshastigheden } Vækstenergien.

De to første Spørgsmaal er behandlet i Arbejdets første Halvdel, det sids i anden Halvdel.

Resultaterne er følgende:

Ad 1. En direkte dræbende Virkning paa Kræftcellerne opnaar man før ved Doser der ligger over 6 H. E. D. Den dræbende Dosis er ens for d Sarcom og det Carcinom, jeg har arbejdet med og den samme ved Bestraalin saavel in situ som in vitro.

Ad 2. Mindre Røntgendoser kan hæmme Svulsternes Vækstenergi; det giver sig dels Udslag ved en forlænget Latenstid dels ved en formindsket Væks hastighed.

Ad 3. Det er muligt ved lokal Røntgenbestraaling af Huden at øge Or ganismens Modstandskraft mod senere implanteret Kræftvæv, baade naar dett implanteres intra- og subcutant, mest udtalt i første Tilfælde og ved Anvendels af det mindre virulente Carcinom.

I de Forsøg, hvor Carcinomet er implanteret i bestraalet Væv, viser Virk ningen sig ved Nedsættelse af Anslagsprocenten, Forlængelse af Levetiden efte Implantationen samt ved en Nedsættelse af Svulstvævets Vækstenergi.

I de Forsøg, hvor Sarcomet er implanteret i bestraalet Væv, faar man ku en Nedsættelse af Svulstvævets Vækstenergi, men ingen sikker Virkning pa Levetiden efter Implantationen og ingen Nedsættelse af Anslagsprocenten.

Desuden finder man i disse Tilfælde, at Sarcomvævet hos de bestraaled Dyr viser en langt større Tilbøjelighed til at vokse igennem Thoraxvæggen en hos de ubestraalede. Dette finder Sted i henholdsvis 56 og 3.6 % af Tilfæ dene. Dette Fænomen skyldes utvivlsomt Røntgenbestraalingen, men det er ikk muligt endnu med Sikkerhed nærmere at gøre rede for de Faktorer, der betinge at denne Gennemvækst finder Sted.

SUMMARY

The purpose of this study has been the investigation of the following questions:

1. How large a dose of roentgen must be used in order to obtain a directly lethal effect on cancer cells?

2. Can it be shown that non-lethal doses have any effect on the cancer cells?

3. Is it possible by local roentgen irradiation either to increase or reduce the capacity of the host for resisting a cancer tissue implanted either subcutaneously or intracutaneously in the exposed area?

The experiments were made with mice, a carcinoma and a sarcoma being used, both from our own animals. The carcinoma was a spontaneous carcinoma of the breast; the sarcoma was a result of tar-painting. The latter was the most malignant of the two.

The direct effect of the roentgen rays was studied partly in the way that cancer tissue was irradiated *in vitro* and implanted subcutaneously directly afterwards, partly by irradiating tumors *in situ* and immediately transplanting fragments of them to other mice.

The degree of the roentgen effect was measured by 1) the proportion of takes, 2) the duration of the animal's life after transplantation, 3) the length of the period of latency and 4) the rapidity of growth; the two latter constituting what we have termed the »energy of growth».

The two first questions are dealt with in the first half of the work, the last one in the second half.

The results were as follows:

Ad 1. Only doses of over 6 HED produced a directly lethal effect. In the case of the two tumors used in these experiments, the lethal dose was the same for the sarcoma and the carcinoma, and the same whether the tumor was irradiated *in vitro* or *in situ*.

Ad 2. Smaller doses lessened the energy of the tumor's growth. This inhibitory effect showed itself partly in a lengthening of the period of latency, partly in a diminished rapidity of development.

Ad 3. It was found that preliminary irradiation of the skin increases the power of the organism to resist a tumor subsequently implanted either subcutaneously or intracutaneously in the exposed region. The effect was most distinctly noticeable when the implantation had been done subcutaneously, and in the case of the less virulent carcinoma.

In the case of the sarcoma, only the energy of the tumor's growth was reduced, while there was no positive effect on the duration of its life after implantation, and no reduction in the proportion of takes.

Also it was found, in these cases of the sarcoma, that the neoplastic tissue had a far greater tendency to grow through the thoracic wall in the irradiated animals than in the non-irradiated ones; such invasive growth being observed in 56 per cent. of the former, but only in 3.6 per cent. of the latter. This must undoubtedly be ascribed to the effect of the irradiation; but we are not able, as yet, to determine with any degree of certainty what factors exactly are responsible for the phenomenon.

RÉSUMÉ

Dans ce travail nous avons voulu examiner les questions suivantes:

1. Quelle dose de rayons roentgen faut-il employer pour obtenir un eff directement mortel sur les cellules cancéreuses?

2. Peut-on démontrer que les doses non-mortelles de rayons roentgen c un effet quelconque sur les cellules cancéreuses?

3. Peut-on, par irradiation locale avec des rayons roentgen, soit augmente soit diminuer, la résistance qu'opposera l'organisme-hôte contre une tume cancéreuse implantée — dans ou sous la peau — dans le champ ainsi irradié?

Les expériences furent faites sur des souris, en employant un carcinome un sarcome. Le sarcome était le plus malin des deux; l'un et l'autre provenaie de nos propres animaux.

Le carcinome était un carcinome spontané du sein; le sarcome était surve comme résultat du goudronnage de la peau.

L'étude de l'action directe des rayons roentgen sur les cellules cancéreuses fit en partie de la manière qu'on irradiait le tissu néoplasique *in vitro,* l'implanta sous la peau immédiatement après; en partie en irradiant *in situ* des tumeu dont on transplantait ensuite des morceaux, immédiatement, à d'autres sour

L'effet fut mesuré par 1) la proportion des prises, 2) le temps que vivaie les souris après avoir été inoculées, 3) la longueur de la vie latente du tumeu et 4) la rapidité de son développement; ces deux derniers facteurs constituant que nous appelons: l'énergie de croissance.

Les deux premières questions sont traitées dans la première moitié l'ouvrage; la troisième dans la dernière partie de celui-ci.

Les résultats de nos observations sont les suivants:

Ad 1. Seulement les doses supérieures à 6 unités H ont un effet directemer mortel sur les cellules cancéreuses. Pour le sarcome et le carcinome avec lesque nous avons opéré, la dose mortelle était la même; et cela indifféremment de que les tumeurs furent irradiées *in situ* ou *in vitro.*

Ad 2. Les doses moindres peuvent retarder l'énergie de croissance des tu meurs; ce retard se traduisant en partie par une prolongation de leur vie latent en partie par une diminution de la rapidité de leur développement.

Ad 3. L'irradition locale de la peau augmente la résistance qu'opposer l'organisme contre une tumeur subséquemment implantée soit sous la peau, so dans la peau même. L'effet est le plus fortement marqué quand c'est la deuxièm de ces méthodes qu'on emploie, et que la tumeur implantée est le carcinome, moi virulent. Dans le cas du carcinome, l'effet se traduit par une réduction d pourcentage des prises, prolongation de la vie latente après implantation, diminution de l'énergie de croissance du tissu néoplasique. Dans le cas d sarcome, c'est seulement la diminution de l'énergie de croissance du tissu néoplas que qu'on observe; tandis qu'il n'y a aucun effet sûr soit sur la durée de la v latente, soit sur le pourcentage des prises.

Dans ces cas du sarcome nous remarquons aussi que la tendance du tiss néoplasique à se propager à travers la paroi thoracique est de beaucoup plus grand dans les souris préalablement irradiées que dans les autres, non-irradiées. Cet propagation à travers la paroi thoracique, nous l'avons observée dans 56 pou cent des uns et seulement dans 3.6 pour cent des autres. Il n'y a pas de doute qu le phénomène ne doit être attribué à l'irradiation; mais pour ce qui est des facteu qui le déterminent, nous ne saurions pas encore exprimer une opinion bien précis

ZUSAMMENFASSUNG

Der Zweck der vorliegenden Arbeit war die Untersuchung folgender Fragen:

1. Eine wie grosse Röntgendosis ist erforderlich, um eine direkt tödliche Wirkung auf Krebszellen zu erzielen?

2. Kann man nachweisen, dass nicht tödliche Dosen einen Einfluss auf Krebszellen haben?

3. Ist es möglich, die Widerstandskraft eines Wirtsorganismus gegenüber intra- oder subkutan im bestrahlten Gebiete implantiertem Krebsgewebe durch lokale Röntgenbestrahlung zu erhöhen oder herabzusetzen?

Die Versuchstiere waren Mäuse, und es wurden ein Carcinom und ein Sarkom benutzt, von denen das Sarkom, das bösartigste ist; beide sind bei meinen eigenen Tieren entstanden. Das Carcinom ist ein spontanes Mammacarcinom. Das Sarkom ist nach Teerpinselung der Haut entstanden.

Die direkte Wirkung der Röntgenstrahlen auf die Krebszellen wurde teils durch Bestrahlung des Geschwulstgewebes in vitro, teils durch sogleich nach der Bestrahlung erfolgte subkutane Implantation desselben, und teils endlich durch Bestrahlung der Geschwülste in situ und unmittelbar nach der Bestrahlung ausgeführte Transplantation von Stücken derselben auf andere Tiere untersucht.

Als Mass für den Grad der Einwirkung wurde benutzt: 1. das Anschlagsprozent, 2. die Lebensdauer der Tiere nach der Transplantation, 3. die Latenzzeit und die Wachstumsgeschwindigkeit = die Wachstumsenergie.

Die ersten beiden Fragen wurden in der ersten Hälfte der Arbeit behandelt, die letzte in der zweiten Hälfte.

Ad 1. Eine direkt tödliche Wirkung auf die Krebszellen erzielt man erst durch Dosen die über 6 H. E. D. liegen. In bezug auf das von mir benutzte Sarkom und Carcinom ist die Dosis die gleiche, und zwar sowohl bei Bestrahlung in situ als auch in vitro.

Ad 2 Kleinere Röntgendosen vermögen die Wachstumsenergie der Geschwülste zu hemmen, dies tritt teils durch eine verlängerte Latenzzeit, teils durch eine verminderte Wachstumsgeschwindigkeit in Erscheinung.

Ad 3. Es ist möglich, die Widerstandskraft des Organismus gegen später sowohl intra- als auch subkutan implantiertes Krebsgewebe durch lokale Röntgenbestrahlung der Haut zu vermehren, und zwar am ausgesprochensten im ersten Falle und bei Benutzung des weniger virulenten Carcinoms.

In denjenigen Versuchen, wo das Carcinom in bestrahltes Gewebe implantiert ist, zeigt die Wirkung sich durch Herabsetzung des Anschlagsprozenten, Verlängerung der Lebensdauer nach der Implantation sowie durch Herabsetzung der Wachstumsenergie des Geschwulstgewebes.

In denjenigen Versuchen, wo das Sarkom in bestrahltes Gewebe implantiert ist, erzielt man lediglich eine Herabsetzung der Wachstumsenergie des Geschwulstgewebes, aber weder eine sichere Wirkung auf die Lebensdauer nach der Implantation noch eine Herabsetzung des Anschlagsprozenten.

Ausserdem findet man in diesen Fällen, dass das Sarkomgewebe bei den bestrahlten Tieren eine erheblich grössere Neigung hat durch die Thoraxwandung hindurchzuwachsen als bei den unbestrahlten Tieren.

Das geschieht in 56 bezw. 3.6 % der Fälle.

Dies Phänomen ist fraglos auf die Röntgenbestrahlung zurückzuführen, es ist aber bislang nicht möglich gewesen, die Faktoren, durch die das Durchwachsen bedingt wird, einwandfrei festzustellen.

LITERATURE

THE FOLLOWING ABBREVIATIONS ARE USED

A I P = Annales de l'Institut Pasteur.
A J R = The American Journal of Roentgenology and Radium Therapy.
A K C = Archiv für klinische Chirurgie.
A M E A P = Archives de Médicine expérimentale et d'Anatomie pathologique.
A P = Archiv für die gesamte Physiologie.
A R = Acta Radiologica.
B A F C = Bulletin de l'Association Française pour l'Étude du Cancer.
B B = Bruns' Beiträge zur klinischen Chirurgie.
B I P = Bulletin de l'Institut Pasteur.
B J R = The British Journal of Radiology.
B K W = Berliner klinische Wochenschrift.
B L = Bibliotek for Læger.
B M J = The British Medical Journal.
C A S = Comptes rendus de l'Académie des Sciences.
C S B = Comptes rendus de la Société de Biologie.
D M W = Deutsche medicinische Wochenschrift.
D Z = Dermatologische Zeitschrift.
D Z C = Deutsche Zeitschrift für Chirurgie.
F G R = Fortschritte auf dem Gebiete der Röntgenstrahlen.
F M = Fortschritte der Medicin.
H = Hospitalstidende.
J A M A = The Journal of the American Medical Association.
J C R = The Journal of Cancer Research.
J E M = The Journal of Experimental Medicine.
J M F = Le Journal Médical Français.
J P B = The Journal of Pathology and Bacteriology.
J Z K = Japanische Zeitschrift für Krebsforschung.
L = The Lancet.
M K = Medizinische Klinik.
M M W = Münchener medicinische Wochenschrift.
N M L = Norsk Magasin for Lægevidenskaben.
P M = Le Progrès Médical.
P R S L = Proceedings of the Royal Society of London.
P S E M = Proceedings of the Society of Experimental Medicine.
R P S = Riv. di Patologia sperimentale.
S M = La Semaine Médicale.
S R I C R = Scientific Report of the Imperial Cancer Research Fund.
Str. = Strahlentherapie.

U L = Ugeskrift for Læger.
V A = Virchows Archiv.
W K W = Wiener klinische Wochenschrift.
W M B = Wiener medicinische Blätter.
Z B P = Zentralblatt für Bacteriologie und Parasitenkunde.
Z C = Zentralblatt für Chirurgie.
Z E T = Zeitschrift für die gesamte experimentelle Therapie.
Z I = Zeitschrift für Immunitätsforschung.
Z K = Zeitschrift für Krebsforschung.
Z M W = Zentralblatt für die medicinischen Wissenschaften.
Z R = Zentralblatt für die gesamte Radiologie.

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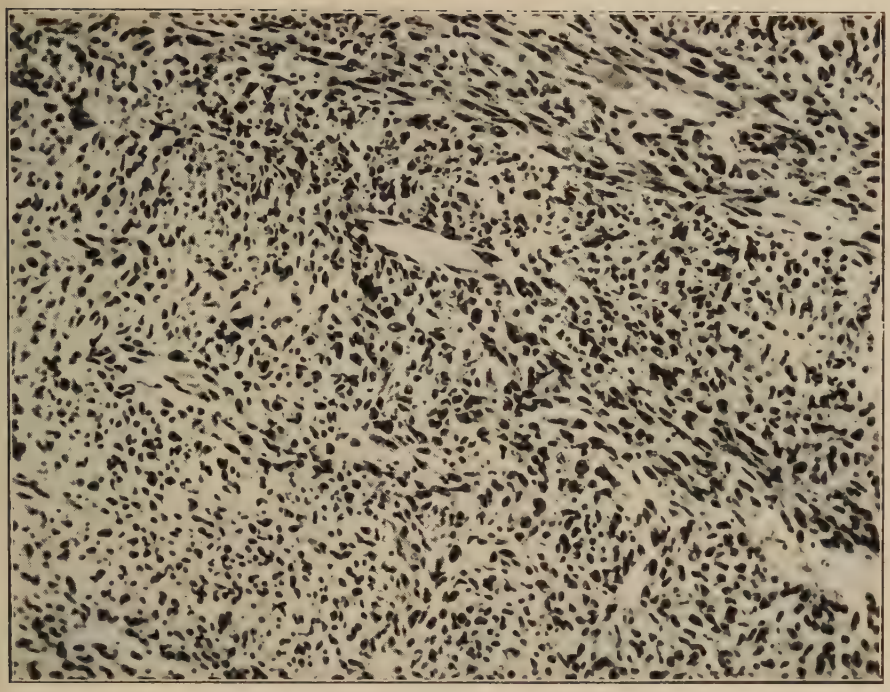
The Translation into English by Robert Fraser.

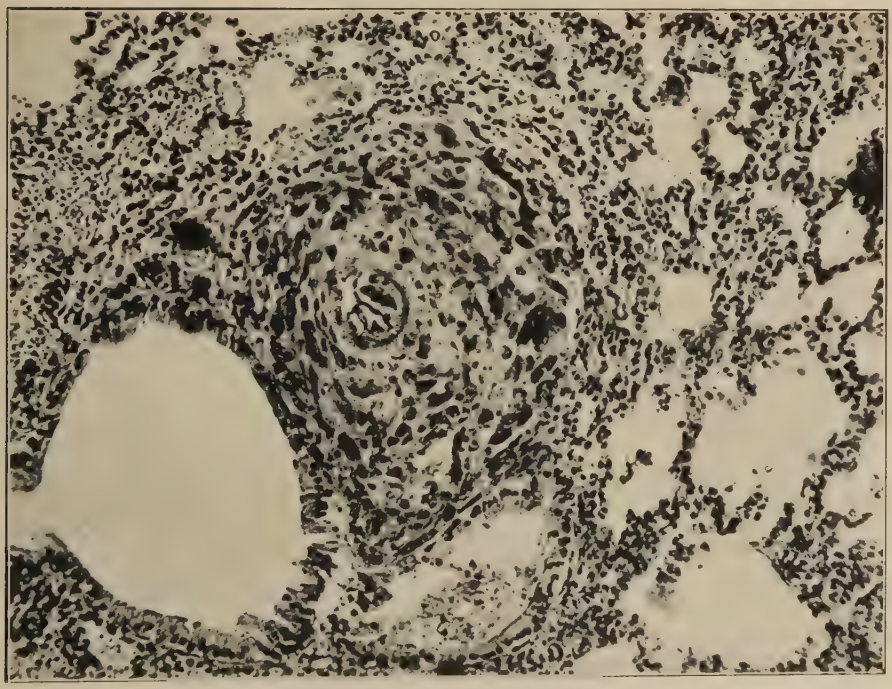
TABULÆ

The Carcinoma

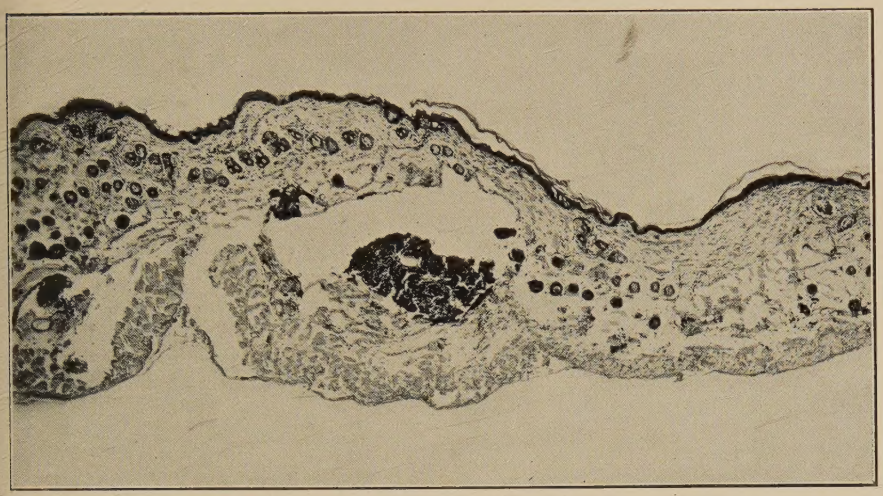
Pulmonary metastasis of the carcinoma.



The Sarcoma.



Pulmonary metastasis of the sarcoma.



Intracutaneous implantation.

Inside of the thoracic wall of a mouse showing ingrowth of tumor.